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JOURNAL OF DAIRY SCIENCE

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THE GROWTH OF *PENICILLIUM ROQUEFORTI* ON SYNTHETIC MEDIA

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INTRODUCTION

The manufacture and ripening of blue veined types of cheese, such as Roquefort, Stilton and Wensleydale, present many difficulties if a high quality of cheese is to be made (18) (4) (21). Thom and Currie (22) show that the dominance of Roquefort mold in cheese is dependent upon a relatively narrow margin of oxygen which gives the right air supply. With Wensleydale cheese made under relatively well controlled conditions, Golding (10) has shown that the development of the mold is a matter of considerable uncertainty.

The process of manufacture and ripening of blue veined cheese may well be said to be an art and standardized methods cannot be adopted due to the existence of too many variables.

With these considerations in view, it was realized that another and entirely different approach to mold ripened cheese might be possible. The pasteurization or processing of cheese has made particularly rapid strides in the last few years (19). Therefore, where it is possible to develop a satisfactory enzyme concentrate this might be added to the cheese subsequent to heating and the desired flavor develop in the processed cheese.

In considering the above idea, it was appreciated that at first further knowledge must be obtained as to the growth of the mold and conditions that will produce maximum growth in a suitable medium.

Naylor *et al.* (15), working with *P. roqueforti* (Thom) have shown that the largest mold growth obtained produced the greatest enzyme formation. Knapp (14) shows that mold growth and enzyme formation go hand in hand. Further the quantity of a particular enzyme produced is dependent on the medium on which the mold is grown (7) (20) (13).

The particular salts required by the molds when growing in a synthetic medium are those in Czapeks formula modified by A. W. Dox (22) (11).

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That such salts are satisfactory for the growth of *P. roqueforti* has also been shown (15) (11). However, Naylor *et al.* (15) have obtained considerable advantage in growth by replacing $NaNO_3$ by NH_4Cl and by reducing the quantity of NH_4Cl used when casein is included in the medium.

For the organic nutrients of the medium casein and dextrose have been shown to be suitable (11) (15). On the other hand, lactose is not as satisfactory (11).

The optimum temperature of growth is of importance as shown by Knapp (14). The optimum temperature of growth for the culture of *P. roqueforti* used in these experiments has been shown to be between 20.6° C. and 28.9° C. (11).

The initial reaction of the media for the growth of *P. roqueforti* was considered by Naylor *et al.* (15). The change in reaction of media produced by the growth of microorganisms has been investigated (12) (3) (6) (8) (1). In the presence of sugar the reaction is changed to the acid side (3) (6) (8) (1) and later to the alkaline side (3) (6) (1).

The wide range of pH possible during growth of mold as shown by Johnson (12) presents a problem as to the type of proteolytic enzymes likely to be elaborated. Buchanan and Fulmer (5) show pepsinases are most active at pH 2.0 to 5.0, tryptases at pH 7.0 to 9.0. Dox (7) working with *P. camemberti* attributes the casein digestion as due to a type of "erepsin," and is of the opinion that plant ereptase has its reaction range in the direction of acidity. However, in his summary of the literature Dox (7) states: "The nature of the protease has not been established, though most of the authors consider it as closely resembling trypsin."

As to whether the protease elaborated by molds is extra or intracellular would appear to be limited by the stage of growth as the greatest enzyme action is found in the mold filtrate when sporulation is well advanced (7) (15). The secretion of enzymes into the culture medium does not ordinarily occur to any appreciable extent during the active vegetative period of the mold. However, Buchanan and Fulmer (5) are of the opinion that the reaction of the medium may be important in determining whether an enzyme will pass the filter.

MATERIALS AND METHODS

Culture of Mold. In all the work done in this investigation a culture of *P. roqueforti* designated No. 32 was used. This culture is described under No. 16 in (11) and its casein digestion is given in (10) under No. 32. Since isolation, the culture has been retained in our mold culture collection on potato or wort agar. Transfers have been made twice each year.

Growth for Inoculation. When required for any experiments fresh cultures were grown on potato or wort agar and the inoculum obtained from the well developed slant into 100 ml. of distilled water. One ml. of this water was used to inoculate each flask.

MEDIA

Salts. The salts used in preparing all the synthetic media for each 1000 ml. of final medium were:

$MgSO_4$	.50	gram
K_2HPO_4	1.00	"
KCl	.50	"
$FeSO_4$	.01	"
NH_4Cl (0.03 N)	1.61	"

These salts in the above proportions were used by Naylor *et al.* (15).

Butterfat. The butterfat when used was obtained from fresh, sweet cream butter. This fat was emulsified into the medium by the use of a small hand power Hurcol emulsifier.

Casein. Will Corporation (Rochester, N. Y.) acid casein was used in all the experiments and was obtained in solution by the method adapted by S. Henry Ayres (2), the amount of NaOH and HCl being varied according to the percentage of casein used. The final reaction of the medium was adjusted to a pH between 6.20 and 6.00.

Dextrose. Difco bacto dextrose was used in the proportions as stated, being added to the medium before it was made up to volume.

$CaCO_3$. Where $CaCO_3$ was used 0.2 grams of precipitated chalk were weighed separately into each flask.

Media Flasks. The medium was added in measured quantities of 25 ml. to each 125 ml. Erlenmeyer flask, which was then plugged and sterilized at 12 pounds pressure for 20 minutes. For the nitrogen determination each flask was weighed to the second decimal place so that the loss by evaporation could be made up before filtering.

Hydrogen-ion Determination. All pH determinations were made at 25° C. using quinhydrone, a gold electrode and a Leeds and Northrup potentiometer No. 7654. The determinations in every case were made on the undiluted filtrate.

Quantitative Determinations for Sugar. The quantitative determinations for dextrose were made with Fehling's solution.

In several cases where considerable sugar is recorded by the above method quantitative determinations by the Schaffer Hartmann method did not show more than 0.3 per cent. When using a medium of 15 to 20 per cent dextrose as much as 0.3 per cent might come from the part which unavoidably dries on the inside of the flask and, therefore, cannot be reached by the mold.

Felt Weight. The felt from each flask, at the required age, was filtered off into weighed Gooch crucibles. The filtrate was collected for pH, sugar, and nitrogen determinations. The felts were washed with distilled water, dried at 100° C. till constant weight. The weight of dry matter does not

express the exact weight of mycelium due to some precipitate being thrown down in sterilization and also to the precipitation of the casein by the acid formed. However, these weights are comparable and when expressed with the pH of the filtrate a satisfactory interpretation can be made.

Nitrogen Distribution in Filtrate. The 125 ml. Erlenmeyer flasks, both the control and those in which the mold had grown, were made up to weight with distilled water and filtered. Sufficient flasks were taken to provide about 230 ml. of the required filtrate. The total nitrogen of the filtrate was determined on 5 ml. lots in triplicate. The protein breakdown was determined according to the methods described by Eagles and Sadler (9) for the following:

1. The soluble nitrogen (acetic acid filtrate).
2. The amino nitrogen (phospho-tungstic filtrate).
3. The proteose nitrogen (sodium sulphate precipitate).
4. The peptone nitrogen (tannic acid precipitate).
5. The sub-peptone nitrogen (tannic acid filtrate).

One and two were adapted from the methods of Orla-Jensen (16) (17) and three, four and five from the methods of Wasteneys and Borsook (23).

EXPERIMENTAL

The Growth of P. roqueforti on the Standard Salt Medium Plus Two Per Cent Butterfat and One Per Cent Casein and Various Percentages of Dextrose

The 125 ml. flasks containing 25 ml. of the above medium with nil, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 per cent of dextrose respectively were inoculated and grown for a period of ten days at 23° C.

The results obtained, given in table I, show:

1. The weight of dry felt produced (whether extracted with ether or not) increases with the increased percentage of dextrose in the medium.

2. With nil and 2.5 per cent dextrose the filtrate had been changed towards the alkaline side. In all other flasks the reaction had been changed more to the acid side.

3. The greater the percentage of dextrose in the medium the higher the acidity produced in the filtrate.

The change in reaction of the filtrate due to growth is given on the 4, 6, 8 and 10th days respectively. From these determinations it is seen that there is a close relationship between the presence of dextrose and the reaction of the filtrate. As soon as the dextrose is used up by the growth of the *P. roqueforti* the reaction of the filtrate gradually moves towards the neutral. No acidity is produced in the medium without dextrose.

The Growth of P. roqueforti in the Standard Salt Medium Plus Various Percentages of Casein and Dextrose

The standard 125 ml. flasks containing 25 ml. of the medium, 1 per cent casein and 2.5, 3.0, 4.0, 5.0, 6.0 and 7.0 per cent of dextrose respectively together with flasks of 2 per cent casein and 8 and 10 per cent of dextrose respectively were inoculated and incubated at 22° C.

The weight of dry felt, the pH of the filtrate and the presence or absence of sugar are given in table II which shows:

1. The amount of growth of *P. roqueforti*, as expressed by the weight of felt, is dependent upon the percentage of casein and dextrose. Further an excess of dextrose over casein, beyond a certain point (about in the ratio of 5 to 1) does not materially increase the growth.

2. The maximum felt weight is obtained during the growth of *P. roqueforti* in the medium when all the sugar is used and from then on the weight of felt decreases.

3. The proportion of dextrose to casein is an important factor in determining the reaction of the filtrate during the growth of *P. roqueforti*.

4. As soon as the dextrose is used up by the mold the reaction of the filtrate tends to approach neutral.

5. In the case of the medium containing 1 per cent casein and 7 per cent dextrose, the casein would appear to be insufficient to allow the growth of mold to use up all the dextrose.

In the case of the 2 per cent casein and 8 per cent dextrose, the difference is striking; here the dextrose is almost all used up by the 12th day and the filtrate is changed towards neutral.

The Growth of P. roqueforti on the Standard Salt Medium with High Percentage of Casein and Dextrose

The object of this experiment was to determine how far increased growth as measured by weight of felt could be obtained by increasing the percentage of casein and dextrose in the medium in the right proportion. Erlenmeyer flasks of 125 ml. with 25 ml. of the medium containing the same standard salts were prepared with various percentages of casein and dextrose (see table III). These were inoculated with *P. roqueforti* and incubated at 20° C. with a high relative humidity in the incubator.

The pH of the filtrate, the weights of dry felt and the presence or absence of sugar are given in table III.

1. The growth as shown by the weight of felt increases with the percentage of casein and dextrose.

2. The time required to obtain the maximum felt weights is in proportion to the percentage of nutrients. A reduction in the weight of felt after the 14th day with mold growing in media of 10 to 13 per cent dextrose and growth increase with the remaining flasks of higher concentration until the 17th day. After the 17th day all felts show a reduction in weight.

TABLE I

Growth of P. roqueforti (Culture 32) on Standard Salt Medium + 2% Butter Fat; 1% Casein and Various % Dextrose

Incubated at 23° C. Inoculated October 18, 1929

DAYS GROWTH	4		6		8		10			
% Dextrose	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	Weight of dry felt	Weight of dry extracted felt
Nil	6.05	Nil	6.80	Nil	7.05	Nil	7.25	Nil	.46	.21
2.5	3.40	Present	6.10	Nil	6.70	Nil	6.75	Nil	.76	.47
3.0	3.40	Present	4.70	Nil	6.35	Nil	5.50	Nil	.89	.56
3.5	3.30	Present	3.45	Trace	5.80	Nil	5.55	Nil	.92	.56
4.0	3.20	Present	2.55	Trace	4.20	Nil	5.35	Nil	.97	.56
4.5	3.00	Present	2.55	Trace	2.85	Trace	3.70	Nil	1.05	.57
5.0	3.05	Present	2.25	Present	2.50	Present	2.20	Nil	1.10	.63

TABLE II

Growth of P. roqueforti (Culture 32) in Standard Salt Medium + Various % of Casein and Dextrose

Incubated at 22° C. — Inoculated November 22, 1929

DAYS GROWTH		10			12			14			16			18	
% Casein	% Dextrose	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	pH	Weight of dry felt
1	2.50	7.70	.34	Nil	7.85	.32	Nil	7.90	.31	Nil					
1	3.00	7.40	.40	Nil	7.70	.38	Nil	7.85	.37	Nil					
1	4.00	6.30	.51	Nil	7.25	.47	Nil	7.25	.43	Nil					
1	5.00	2.90	.62	Trace	2.65	.57	Nil	2.50	.53	Trace					
1	6.00	2.25	.62	Present	2.05	.60	Present	2.25	.64	Trace					
1	7.00	2.05	.63	Present	2.05	.62	Present	2.10	.68	Present					
2	8.00	3.80	.86	Present	5.45	.89	Trace	6.70	.89	Nil	6.95	.82	Trace	7.40	.79
2	10.00	3.15	.92	Present	3.70	1.10	Present	5.75	1.10	Nil	5.85	1.14	Trace	6.55	1.03

TABLE III

Growth of P. roqueforti (Culture 32) on Standard Salt Medium with high Percentages of Casein and Dextrose

Incubated at 20° C.										Inoculated December 20, 1929
DAYS GROWTH		14 DAYS			17 DAYS			20 DAYS		
Casein %	Dextrose %	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar	pH	Weight of dry felt	Sugar
2	10	5.25	1.09	Slight	6.00	1.09	Trace	6.70	1.01	Nil
3	11	6.45	1.30	Considerable	7.35	1.25	Trace	7.55	1.20	Nil
3	12	6.80	1.37	Slight	7.25	1.36	Trace	7.35	1.34	Trace
3	13	6.30	1.49	Heavy	7.15	1.38	Trace	6.85	1.31	Slight
3	14	5.05	1.44	Very heavy	6.75	1.53	Slight	7.00	1.48	Slight
4	15	7.20	1.65	Considerable	7.70	1.73	Slight	7.70	1.63	Considerable
4	16	6.60	1.58	Considerable	7.55	1.76	Slight	7.60	1.69	Considerable
4	17	5.75	1.66	Heavy	7.50	1.87	Slight	7.65	1.72	Considerable
4	18	5.10	1.77	Heavy	7.40	1.97	Slight	7.50	1.83	Considerable
5	19	5.05	1.80	Heavy	7.60	2.00	Considerable	7.70	1.89	Considerable
5	20	4.90	1.81	Heavy	7.40	2.11	Heavy	7.70	1.92	Considerable

TABLE IV

Protein breakdown of casein by P. roqueforti (Culture 32) at different hydrogen-ion concentrations

EXPERIMENT	1		2			3			4		
Standard Salt Media +	Casein 3% Dextrose 18% $CaCO_3$ .8%		Casein 3% Dextrose 18% $CaCO_3$ .8%			Casein 2% Dextrose 16% $CaCO_3$ Nil			Casein 2% Dextrose 16% $CaCO_3$ Nil		
Incubation temp.	23.0° C. (Approx.)		24.0° C.			24.0° C.			22.5° C.		
Inoculation	Control	Inoculated	Control	Inoculated		Control	Inoculated		Control	Inoculated	
Days growth	Nil	9	Nil	10	14	Nil	10	14	Nil	11	18
pH of filtrate	6.20	6.35	6.05	6.55	7.25	6.15	3.20	2.80	6.25	3.00	3.20
	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. Nitrogen in 100 ml.	Mg. in 100 ml. Nitrogen
Soluble nitrogen (water soluble)	403.0	107.0	378.0	64.0	74.0	274	82.0	56.0	291.2	63.0	61.4
Proteose nitrogen (sodium sulphate precipitate)		8.20	1.48	1.74	6.96		6.28	7.32	.53	1.48	2.81
Peptone nitrogen (tannic acid precipitate)		45.64	16.36	29.20	18.58		34.96	24.32	6.63	30.62	24.33
Sub. peptone nitrogen (tannic acid filtrate)		42.16	42.16	26.66	45.26		23.56	23.56	42.04	27.90	32.86
Filtrate 3A { Proteose Peptone Sub. peptone	55.2	96.0	60.0	57.6	70.8	52.0	64.8	55.2	49.2	60.0	60.0
Soluble nitrogen (acetic acid filtrate)	75.0	100.5		60.5	75.5	68.0	78.0	59.0	66.2	62.0	60.3
"Amino" nitrogen phospho-tungstic filtrate	15.0	43.5	24.0	30.0	36.0	24.0	33.0	24.0	15.9	32.1	35.25

3. The pH of the filtrate, shows that in all cases during the latter stages of growth the medium is returning from acid to neutral or alkaline reaction. This change is more marked in the flasks showing growth between the 14th and 17th day.

4. The presence or absence of dextrose in the medium after growth shows the same general trend as in the previous experiments. However, the considerable quantity of dextrose, which attaches to the sides of the Erlenmeyer flasks when preparing and sterilizing media of such high dextrose concentrations, makes a qualitative determination of uncertain value.

5. *P. roqueforti* is capable of growing well in media with high concentrations of casein and dextrose. The dry felts obtained from such growth amount to as much as 8 per cent of the media.

The Protein Breakdown of Casein by P. roqueforti at Different Hydrogen-ion Concentrations

From the previous experiments it is seen that the hydrogen-ion concentration during growth could be considerably varied. With the production of an enzyme extract in mind it was considered desirable to determine the difference in the protein breakdown during growth under variable conditions of pH. Two media were planned, namely, (1) standard salts; 3 per cent casein; 18 per cent dextrose, and 0.8 per cent $CaCO_3$ to maintain a reaction around neutral during growth. (2) Standard salts; 2 per cent casein; 16 per cent dextrose to produce and maintain a strongly acid reaction during growth.

Figure 1 shows the change of reaction of these two media during the growth of *P. roqueforti* and the final reaction on the day for analysis is given in table IV. Both show the desired reaction was obtained.

The Erlenmeyer flasks of 125 ml. were prepared with 25 ml. of medium in each as stated. The weight of each flask was recorded before sterilization so that loss by evaporation could be made with distilled water just before analysis. After sterilization the flasks were inoculated and incubated as stated in table IV. At the end of the growth period the flasks were made up to their original weight with water and filtered.

The filtrate was analyzed for the distribution of nitrogen and though the data are not extensive the following trends are shown in table IV:

1. By comparison with the soluble nitrogen in the control it is seen that four to six times as much nitrogen is present in the felt as in the filtrate.

2. Considering the higher percentage of casein in the media of Experiment 2, a higher percentage of the total soluble nitrogen is found in the acid filtrates.

3. The soluble nitrogen in Experiment 2 (nearly neutral reaction) increased with a longer period of growth, though the amount in each case is less than the control. The opposite is found to be the case where the mold is growing in an acid medium. (Experiments 3 and 4).

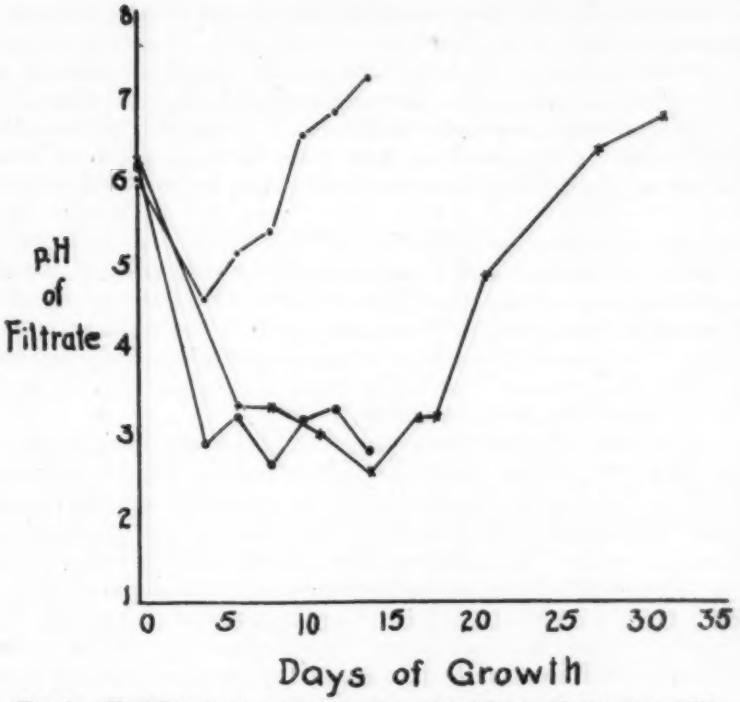

FIG. 1. pH of Filtrate at various ages of growth of *P. roqueforti* (culture 32).
. Experiment 2; Table IV; Casein 3%; Dextrose 18%; $CaCO_3$, .8%
O Experiment 3; " IV; " 2%; " 16%; " Nil.
× Experiment 4; " IV; " 2%; " 16%; " Nil.

4. The proteose nitrogen is somewhat greater in the filtrate where growth has taken place. This figure is increased by a longer period of growth.

5. The amount of proteose nitrogen is not sufficient to conclude a marked difference between the very acid and almost neutral filtrate.

6. The peptone nitrogen shows a definite increase over the control due to the growth of *P. roqueforti*. This increase is reduced with the longer period of growth.

7. With the peptone nitrogen there is no significant difference in the results obtained between the very acid and the almost neutral filtrates.

8. The sub-peptone nitrogen shows a definite decrease from the control in the earlier growth at 10 and 11 days. In the case of Experments 2 and 4 this was increased when the analysis was made at a later period of growth.

9. The sub-peptone nitrogen increased much more rapidly by the growth of *P. roqueforti* in the almost neutral medium than in the acid filtrate.

10. The amino nitrogen, phosphotungstic filtrate shows a tendency to increase over that of the control.

11. From the small amount of data given it would appear that the pH of the medium during growth did not materially change the type of the protein breakdown of casein by the growth of *P. roqueforti* for the growth period studied. However, there is an indication that the protein breakdown would be materially changed were the period for growth extended.

DISCUSSION OF RESULTS

The work reported in this paper shows that with suitable salts and organic nutrients of casein and dextrose a very abundant growth of *P. roqueforti* can be obtained. The maximum growth was dependent on having the casein and dextrose in the right proportion, the mold requiring about six times as much dextrose as casein. A medium of about 25 per cent solids gave the highest felt weight.

As has been previously shown by other workers with bacteria (6) (3) and molds (12) (1) the reaction of the medium was changed during the growth of *P. roqueforti*. This change of reaction may be reversible, depending on the composition of the medium. The growth of *P. roqueforti* on the casein medium free from dextrose causes the reaction to go to the alkaline side. The growth of *P. roqueforti* on the casein, dextrose media causes an acid reaction to develop till all the dextrose is used up, the reaction then reversing towards the alkaline side. The extreme range of pH in these experiments was between pH 2.2 to pH 7.7. However, the change of pH of the media was limited in some cases by the use of precipitated chalk or a suitable balance between dextrose and casein, or a combination of both.

In a study of the protein breakdown of casein under conditions of high and low pH, protein fractionation of the filtrate showed that the subpeptone nitrogen was formed much more rapidly by the growth of *P. roqueforti* in the almost neutral medium than in the acid medium. Other protein fractions did not show marked differences.

The enzymes elaborated by *P. roqueforti* in these experiments have not been studied but according to the investigations of Naylor *et al.* (15) there would be very considerable enzyme formation. However, the obtaining of an enzyme concentrate suitable for commercial application to the processed cheese industry will undoubtedly require very considerable investigation before its application can hope to be recommended.

SUMMARY

With the object in view of obtaining a strong enzyme extract to produce a Roquefort flavor in processed cheese subsequent to heating, methods for obtaining a heavy growth of the mold on synthetic media have been investigated. A specially heavy growth of the mold *P. roqueforti* was obtained

by using a modified Dox (7) salt solution and increasing the proportion of casein and dextrose. Maximum felts were obtained at 5 per cent casein and 20 per cent dextrose.

The change of pH during growth is most extensive, ranging from pH 2.05 to 7.7 depending on the proportion of dextrose and casein. While the dextrose was present in the medium an acid reaction was obtained. The reaction moved towards the alkaline side as soon as the dextrose was used up by the mold.

A preliminary study of the protein breakdown of the casein in the media under widely different hydrogen ion concentrations was made. Thus far it would appear that the pH of the medium during growth did not materially change the type of the general protein breakdown of the casein by the growth of *P. roqueforti* for the growth periods studied.

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A COMPARISON OF THE LEUCOCYTE COUNT, THE BROM THYMOL BLUE REACTION AND THE CATALASE CONTENT OF FRESHLY DRAWN MILK

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INTRODUCTION

The distinguishing characteristics between normal and abnormal freshly drawn milk constitutes a study that has received a renewed interest during the past few years. The results of recent investigations have thrown considerable doubt upon certain of the earlier studies in which milk samples classed as normal and drawn from apparently normal udders were used. In the light of our present knowledge it appears very probable that a considerable percentage of the milk samples from such udders would have given positive reactions to certain chemical tests recognized today as indicating abnormal udder conditions.

Udall and Johnson (14), Hucker, Trudell and Jennings (7), Hucker and Udall (8), and Hucker (9) have emphasized the necessity of ascertaining the physical condition of the udder from which the milk is drawn before classifying it as coming from a normal udder or as being a normal product. These investigators have shown that "apparently normal udders" may show signs of infections in the form of indurations or fibrotic tissue and that the milk coming from such quarters may be abnormal in reaction, in the amount of catalase present and in the chloride and lactose content.

The prevailing conception of the numbers of leucocytes present in normal milk drawn from apparently healthy udders is based upon studies in which the samples were considered normal by their physical properties only. Chemical determinations were not considered necessary in order to establish the normal or abnormal status of these milks.

The object of the present study was to secure information on the leucocyte content of milk in relation to the pH value, the catalase content and to the presence or absence in the producing animal of pronounced positive evidence of mastitis during a period of several months duration.

HISTORICAL

Among the early reports on the leucocyte content of normal milk are those of Cooledge (4), Breed (2), Tweed (13) and Copeland and Olson (5). The average numbers per cc. reported by these investigators were 930,000; 868,000; 626,000; and 657,000 respectively.

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Cherrington, Hansen and Halversen (3) reported the leucocyte count of milk from six normal cows to be 43,000 per cc. as compared to 3,000,000 per cc. for the milk from seven cows known to be suffering from mastitis. They concluded that milk from normal udders usually contains less than 50,000 cells per cc. whereas milk from infected udders almost invariably contains more than 100,000 per cc. Hucker, Trudell and Jennings (7) made an extensive study of the various methods for the diagnosis of mastitis. They reported that no significance could be attached to the leucocyte content when it was less than 3,000,000 per cc. In a more recent study Hucker and Udall (8) found that a cell count of more than 500,000 per cc. always indicated an abnormal or pathological condition of the udder. Still more recently Hucker (9) states that all quarters showing cell counts of more than 150,000 per cc. should be considered as suspicious, if not definitely proved to be infected. He found no quarter free from indurations or fibrotic tissue to discharge more than 150,000 leucocytes per cc. Ninety-nine per cent of the quarters free from scar tissue produced milk having less than 60,000 per cc.

Wayne and Macy (15) recently reported an average cell count of 1,252,000 per cc. for 284 samples of normal milk. They made no statement relative to their methods of determining the normal status of their samples.

During the past few years numerous studies have been made of the hydrogen-ion concentration of milk, especially as it relates to the diagnosis of mastitis and to the detection of milk from infected udders. Baker and Van Slyke (1) developed a colorimetric method for the detection of abnormal milk in which brom cresol purple was used as the indicator to determine the hydrogen-ion concentration. In recent years brom thymol blue has replaced brom cresol purple. This determination is known as the brom thymol blue test, commonly called the thybromol test.

Rosell (12) and Udall and Johnson (14) have made extensive studies on the value of the brom thymol blue test as a means of diagnosing mastitis. Rosell (12) considers it to be one of the most useful and easily applied methods. He found it to be approximately 90 per cent accurate. Udall and Johnson (14) state that the presence of a definite color change always means mastitis when applied to the milk of cows that are not in the advanced stages of lactation or that have not just freshened. They consider it a highly reliable test for the diagnosis of chronic mastitis. Hucker, Trudell and Jennings (7) found that positive reactions to the brom thymol blue test always indicated an infected quarter, but negative results did not assure a normal udder. However, they considered an endpoint of pH 7.0 to represent the dividing line between a normal and an abnormal reaction.

Milk from an infected quarter usually has a higher content of the enzyme, catalase, than does milk from a healthy udder. Gratz and Naray (6) were among the early investigators to report on the value of the catalase test in detecting mastitis. They found the test to be accurate if combined with the leucocyte count. Such was necessary in order to exclude the presence of blood as the cause of the increased catalase content. Hucker, Trudell and Jennings (7) found the catalase test to be an exceedingly delicate method of selecting cows that carry an active udder infection. Hucker (9) reported the catalase test to be highly efficient in detecting both clinical and sub-clinical mastitis. The increase over that of normal usually varies with the extent of the infection.

METHODS

The data presented in this paper represent a comparative study of the brom thymol blue test, the catalase test and the leucocyte count of 1019 samples of freshly drawn milk from individual udder quarters of 40 cows. Samples were collected from most of the animals at intervals varying from one to three weeks and extending over a period of four to five months. Milk from cows in the advanced stages of lactation, during the last three months of the milking period, or that had just freshened was excluded from this study. Such milk usually has a decreased hydrogen-ion concentration and an increased catalase and leucocyte content. These changes are due to physiological rather than to pathological conditions.

The milk samples used in this study were samples of the fore milk and were drawn after the initial four or five streams had been discarded. They were brought to the laboratory immediately for examination.

Leucocyte counts were made by the direct microscopic method as developed by Prescott and Breed (11). Newman's (10) formula No. 2 was used in staining the preparations. Twenty fields of each smear were counted.

The brom thymol blue test was made by adding 1.0 cc. of a 0.04 per cent aqueous solution of the indicator to 5.0 cc. of milk. Normal fresh milk gives a yellowish-green color and has a pH value 6.3 to 6.8. Milk drawn from infected udders usually turns light green, green or dark green, depending on the activity of the inflammatory process. Such milk has a pH value greater than 6.8.

The catalase test was made by mixing 15 cc. of fresh milk and 5.0 cc. of a 1.0 per cent solution of hydrogen peroxide in a fermentation tube. The mixture was incubated at 37 degrees C. for two hours, after which time the amount of liberated oxygen was recorded. The liberation by the catalase of more than 1.5 cc. of oxygen from 5.0 cc. of a 1.0 per cent solution of hydrogen peroxide in 15.0 cc. of freshly drawn milk is generally regarded as indicating an abnormal catalase content.

PRESENTATION OF DATA

The grouping of the samples, based on the leucocyte content, the brom thymol blue and the catalase tests are shown in table 1.

TABLE 1

Leucocyte counts, brom thymol blue reactions and catalase determinations of freshly drawn milk

LEUCOCYTES PER CC.	NO. OF SAMPLES	BROM THYMOL BLUE TEST NORMAL		CATALASE TEST CC. OF LIBERATED OXYGEN					NORMAL TO BOTH BROM THYMOL BLUE AND CATALASE TEST
		No.	%	0–1.5*		1.5–3.0	3.0–5.0	5.0 +	Per cent
				No.	%				
Less than 100,000	513	508	99.0	450	88.5	45	17	1	87.7
100,000–250,000	214	203	94.9	143	66.5	50	18	3	65.5
250,000–500,000	91	72	79.0	36	39.5	28	13	14	37.4
500,000–750,000	58	28	48.4	18	31.0	14	10	16	24.1
750,000–1,000,000	50	22	43.0	4	8.0	22	10	14	8.0
1,000,000–2,000,000	29	5	17.3	1	3.9	2	9	17	.0
Over 2,000,000	64	12	18.7	2	3.1	6	8	48	2.1
Total	1019	850	83.4	654	64.3	167	85	113	63.3

* Normal catalase index.

DISCUSSION OF RESULTS

From a study of table 1, it is seen that a general relation exists between the leucocyte content, and the reaction of the milk samples to the brom thymol blue and catalase tests. As the cellular content increases, there is a corresponding decrease in the percentage of samples giving normal reactions to both these tests. The decrease from normal is more pronounced when measured by the catalase test than when measure by the brom thymol blue test.

Of the samples having leucocyte counts of 250,000–500,000; 500,000–750,000; 750,000–1,000,000; and 1,000,000–2,000,000 per cc., only 79.0 per cent, 48.4 per cent, and 43.0 per cent and 17.3 per cent, respectively, were found to be normal to the brom thymol blue test.

The results secured by the catalase determinations are of interest. Eighty-eight and five-tenths per cent of the samples having leucocyte counts of less than 100,000 per cc. were found to have a catalase index of less than 1.5 cc. of liberated oxygen as compared to 3.9 per cent for the samples ranging in leucocyte count from 1,000,000 to 2,000,000 per cc. In 59 per cent of the samples of the latter group, the catalase index was in excess of 5.0 cc. of liberated oxygen. Samples having leucocyte counts in excess of 2,000,000 per cc. responded to both the catalase and brom thymol blue tests in a manner similar to the samples ranging from 1,000,000–2,000,000 leucocytes per cc.

Eighty-three and four-tenths per cent of the samples gave normal reactions to the brom thymol blue test as compared to 64.3 per cent for the catalase test. Of the 850 samples reacting normal to the brom thymol blue test, only 77 per cent had a normal catalase index. Occasioally a sample was found to give an abnormal reaction to the brom thymol blue test and yet have a catalase index within normal limits. The per cent of samples in each group reacting normal to both tests is shown in the last column of table 1. For the corresponding group of samples, these figures are slightly lower than those for the catalase reaction alone.

The average leucocyte counts of the 850 samples showing negative reactions to the brom thymol blue test and for the 654 samples reacting negatively to the catalase test were 208,000 and 151,000 per cc. respectively. At one time or oftener during the period over which this study extended nine of the animals showed pronounced positive evidence of mastitis as indicated by the production of stringy, or flaky milk that soon separated on standing. Such milk was usually nearly neutral or slightly alkaline in reaction and contained a high cellular content. The average leucocyte content of all samples of milk coming from these nine animals was found to be 2,800,000 per cc. When all samples of milk drawn from such animals were excluded, an average leucocyte content of 225,000 was obtained for 870 samples collected from 122 udder quarters of 31 cows. When all samples of milk from udder quarters, that at any time during the observation period gave milk reacting abnormally to the brom thymol blue test were excluded, an average leucocyte count of 160,000 per cc. was found for 700 samples from 103 udder quarters of 29 cows.

Nineteen cows produced milk throughout the observation period in one or more quarters, that at all times contained a normal catalase content. There were 247 samples in this group coming from 39 udder quarters. The average leucocyte count for these samples was found to be 72,000 per cc. Only four of the nineteen cows produced milk of such low catalase content from all quarters.

SUMMARY AND CONCLUSIONS

A comparative study of the brom thymol blue reaction, the catalase test and the leucocyte content was made of 1019 samples of freshly drawn milk. Milk samples from cows in the advanced stages of lactation or that had just freshened were not included in this study.

Based on the leucocyte content per cc. the samples were grouped as follows: less than 100,000; 100,000–250,000; 250,000–500,00; 500,000–750,000; 750,000–1,000,000; 1,000,000–2,000,000; and over 2,000,000.

In the order of the above increasing leucocyte content, the following percentage of the various groups of samples reacted normally to the brom thymol blue test: 99.0, 94.9, 79.0, 48.4, 43.0, 17.3 and 18.7. In each group a smaller percentage of the samples reacted normally to the catalase test than to the brom thymol blue test. These respective percentages were as follows: 88.5, 66.5, 39.5, 31.0, 8.0, 3.9 and 3.1.

An average leucocyte count of 225,000 was obtained for 870 samples of milk drawn from 31 cows that at all times during the period of observation were free from pronounced positive evidence of mastitis as indicated by the production of stringy or flaky milk. The average cell count of 850 samples reacting negatively to the brom thymol test was 208,000 per cc. as compared to 151,000 for the 654 samples having a catalase index of less than 1.5 cc. The average leucocyte count of 700 samples coming from 103 udder quarters that at all times during the period of observation produced milk giving a normal reaction to the brom thymol blue test was found to be 160,000 per cc. Thirty-nine udder quarters from 19 cows produced milk that at no time showed an excessive catalase content. The average cell count for 247 samples of such milk was found to be 72,000 per cc.

The results of this study indicate that the percentage of animals producing milk, that is normal to both the brom thymol blue and catalase tests, from all quarters and at all times is low.

The average cell count of milk drawn from healthy normal udders is very much less than the average cell count of normal milk as reported by the earlier investigators and by some of the more recent contributors to the subject.

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THE EFFECT OF DAILY MASSIVE DOSES OF VIOSTEROL UPON CALCIUM AND PHOSPHORUS METABOLISM AND BLOOD CALCIUM AND INORGANIC PHOSPHORUS IN CALVES[1]

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The beneficial effect of the administration of normal doses of cod-liver oil or irradiated ergosterol in young calves has been definitely established but the effect of abnormally large doses of irradiated ergosterol in young calves is relatively unknown.

This study was undertaken to observe the possible toxic symptoms and pathologic results produced by excessive daily doses of viosterol in calves on a normal and on a high calcium and phosphorus intake. Calcium and phosphorus balances were determined upon two young healthy calves on a natural ration of whole milk. The blood calcium and inorganic phosphorus values of these same animals were studied together with values obtained from two other young calves on a whole milk ration but on a lower calcium, phosphorus and viosterol intake.

Several observations prior to and since our experiments have indicated that the degree of toxicity of irradiated ergosterol is partially dependent upon the calcium-phosphorus ratio of the diet (1) (2) (3) (4). Young calves are normally on a diet of whole milk in which this ratio is approximately 1 : 1. In order to supply the metabolism animals with an abundance of calcium and phosphorus, the ration was supplemented with enough monocalcium phosphate to bring the total intake of these elements to 15 grams of each per day.

The results of metabolism studies have been reported on rats (5) (6) (7) (8), rabbits (9), dogs and infants (10), therefore an extensive review of this literature is not necessary.

EXPERIMENTAL

Effect of 1000X Viosterol—Preliminary to the metabolism experiment, two young calves (V1 and V2, about 14 days of age) were used to determine the pyramiding effect of heavy viosterol feeding when on a normal diet. Each animal received approximately 10 pounds of milk per day which contained 5.0 grams of calcium and 3.9 grams of phosphorus. In

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addition to the milk they received 25 cc. of Mead Johnson and Company viosterol which had an antirachitic potency of 1000X according to their standardization.[2] The calves were on experiment for 27 days, at which time V2 died as a result of the administration of the viosterol and V1 was slaughtered. Definite overdosage symptoms were not particularly noticeable during the first seven days but after that time the calves became drowsy and were apathetic. It became increasingly difficult to get them to drink the entire 10 pounds of milk and toward the end of the experiment they would not drink more than 6 pounds each per day. At times the feces were very soft and contained some blood but severe diarrhea was not observed.

Blood samples were drawn daily from the jugular vein for 17 days, after which the intervals were lengthened. The blood was analyzed for calcium and inorganic phosphorus by methods used in previous work (11).

At autopsy V2 showed marked hemorrhagic gastritis, V1 displayed the same symptoms but to a lesser degree. The kidneys of both calves were soft and enlarged. The bile sac was removed from each calf, the contents weighed and calcium, phosphorus and magnesium were determined on each sample. The ash content of the right dental pad was determined on V1 and V2 and was found to be 54.1 and 57.7 per cent respectively.

Effect of 2500X Viosterol—The animals used in the metabolism investigation were healthy, young male calves, V3 and V4, approximately 30 days of age. They were placed in individual metabolism stalls and fed the basal ration for five days prior to the oral administration of viosterol. The basal ration consisted of 14 pounds of whole milk, supplemented with enough $CaH_4(PO_4)_2 \cdot H_2O$ to bring the total calcium intake to 15 grams per day. A suspension of 25 grams of the salt was made with about 3 pounds of milk and was given to the calves morning and night. This was followed by feeding 4 pounds of milk. The ration was calculated to supply an excess of calcium and phosphorus to the animals and also to keep the ratio of these elements about 1:1. After a period of five days, 50 cc. of 2500X viosterol was fed to each calf daily.

Feces and urine were collected separately in 24 hour periods. Each daily sample was weighed or measured, thoroughly mixed and sampled for analysis. The excreta were analyzed each day for calcium and phosphorus as in previous work (12). A composite sample of the milk contained 0.1101 per cent calcium and 0.0855 per cent phosphorus.

DISCUSSION OF RESULTS

Blood Calcium and Inorganic Phosphorus

The effects of the two levels of viosterol feeding on the blood pictures of the four calves are shown in table 1. It is of interest to note that these

[2] We are indebted to Mead Johnson and Company for the viosterol used in these experiments.

TABLE 1

The calcium and phosphorus content of the blood, mgm. per 100 cc. of plasma

DATE	V1		V2		DATE	V3		V4	
	Ca	P	Ca	P		Ca	P	Ca	P
May 28*	11.5	6.51	12.1	6.68	Oct. 25*	12.7	6.84	12.2	6.86
29	11.9	6.38	11.9	7.10	26	13.2	5.87	11.9	6.62
30	13.6	6.19	12.6	6.48	27	12.8	6.62	12.5	7.71
31	13.6	7.40	12.3	7.23	28	14.2	7.35	13.1	8.87
June 1	13.0	7.27	13.0	7.44	29	12.8	7.86	12.2	9.13
2	12.3	7.53	12.5	8.12	30	14.4	8.01	12.8	7.96
3	13.1	7.14	12.8	8.17	31	13.0	12.40	12.6	10.20
4	11.8	7.62	12.6	7.35	Nov. 1	Died		14.3	10.95
5	12.2	7.76	12.3	6.95	2			13.9	10.45
6	13.4	8.12	12.2	7.14	3			13.0	9.80
7	12.7	8.01	11.6	5.66	4			12.8	11.10
8	13.0	8.87	11.8	5.19	5			13.1	10.70
9	13.6	8.87	11.4	4.96	6			13.9	9.22
10	12.7	8.23	11.8	5.10	7			12.8	8.90
11	13.6	8.81	12.1	5.81	8			13.8	8.87
12	12.7	7.91	11.9	5.21	9			14.1	9.84
13	13.2	8.01	13.2	5.97	10			10.7	11.54
16	12.2	8.33	11.7	6.65				Killed	
19	10.5	7.62	11.0	7.02					
24	10.8	8.33	10.0	8.01					
	Killed		Died						

* Average of three separate samples prior to feeding viosterol.

young calves did not show marked hypercalcemia when fed large doses of viosterol. However, the blood calcium did increase in a moderate way and had a tendency to reach a maximum value within 72 hours from the time the viosterol was first fed. The pyramiding effect was entirely absent.

The inorganic phosphorus values had a tendency to decrease slightly during the first 24 hours immediately following the first large dose of viosterol. After the initial drop the phosphorus values increased to a maximum but at a slower rate. If the animals remained on experiment for 14 days or longer the blood calcium and phosphorus had a tendency to return to their pre-viosterol feeding values.

The decided terminal drop in the blood calcium curves and the decided increase in the blood phosphorus values of V2 and V3 some hours before death are of interest. V4 displayed the same general blood phenomenon at the time it was destroyed. Collip (13) and Macleod and Taylor (14) have called attention to the terminal decline in the calcium curve caused by parathyroid overdosage in dogs. One of us (15) has previously indicated that the blood calcium curves of calves correspond to this same typical form when fatal overdosage symptoms are produced.

TABLE 2

Calcium and phosphorus metabolism data, animals V3 and V4

DATE	WEIGHT	VIOSTEROL 2500X	CALCIUM						PHOSPHORUS						Ca/P
			Outgo			Intake	Balance	Used	Outgo			Intake	Balance	Used	
			Feces	Urine	Total				Feces	Urine	Total				
Oct.	*pounds*	*cc.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*per cent*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*per cent*	
V3															
21	133	.....	5.98	0.26	6.24	14.93	8.69	58.2	5.25	7.61	12.86	17.73	4.87	27.5	0.84
22	136	.....	7.12	0.15	7.27	14.93	7.66	51.3	5.86	6.85	12.71	17.73	5.02	28.3	0.84
23	133	.....	3.16	0.18	3.34	14.93	11.59	77.6	2.69	6.41	9.10	17.73	8.63	48.7	0.84
24	137	.....	3.19	0.19	3.38	14.93	11.55	77.4	2.37	6.14	8.51	17.73	9.22	52.0	0.84
25	136	.....	7.55	0.16	7.71	14.93	7.22	48.4	5.94	5.83	11.77	17.73	5.96	33.6	0.84
Average	.....	.....	5.40	0.19	5.59	14.93	9.34	62.6	4.42	6.57	10.99	17.73	6.74	38.0	0.84
26	137	50	3.97	0.20	4.17	14.93	10.76	72.1	3.06	6.23	9.29	17.73	8.44	47.6	0.84
27	139	50	9.40	0.21	9.61	13.27	3.66	27.6	7.16	6.29	13.45	16.43	2.98	18.1	0.81
28	135	50	5.20	0.27	5.47	14.93	9.46	63.4	4.03	4.62	8.65	17.73	9.08	51.2	0.84
29	135	50	4.31	0.42	4.73	14.89	10.16	68.2	3.64	5.98	9.62	17.70	8.08	45.6	0.84
30	131	50	7.05	0.33	7.38	14.60	7.22	49.5	3.95	3.94	7.89	17.47	9.58	54.8	0.84
31	132	50	0.56	.....	0.56	10.85	10.29	94.8	0.33	.....	0.33	14.55	14.22	97.8	0.75
Average	.....	.....	5.08	0.29	5.32	13.91	8.59	61.7	3.69	5.41	8.20	16.93	8.73	51.6	0.83
V4															
21	116	.....	6.26	0.17	6.43	14.93	8.50	56.9	4.81	8.18	12.99	17.73	4.74	26.7	0.84
22	120	.....	5.24	0.10	5.34	14.93	9.59	64.2	3.90	6.93	10.83	17.73	6.90	38.9	0.84
23	123	.....	3.36	0.09	3.45	14.93	11.48	76.9	2.19	5.97	8.16	17.73	9.57	54.0	0.84
24	122	.....	2.29	0.10	2.39	14.93	12.54	84.0	1.26	6.23	7.49	17.73	10.24	57.7	0.84
25	125	.....	8.23	0.11	8.34	14.93	6.59	44.2	5.04	6.61	11.65	17.73	6.08	34.3	0.84
Average	.....	.....	5.08	0.11	5.19	14.93	9.74	65.2	3.44	6.78	10.22	17.73	7.51	42.3	0.84

TABLE 2—(*Continued*)

DATE	WEIGHT	VIOSTEROL 2500x	CALCIUM						PHOSPHORUS						Ca/P
			Outgo			Intake	Balance	Used	Outgo			Intake	Balance	Used	
			Feces	Urine	Total				Feces	Urine	Total				
26	126	50	6.05	0.08	6.13	14.93	8.80	59.0	3.68	6.32	10.00	17.73	7.73	43.6	0.84
27	126	50	5.15	0.07	5.22	14.93	9.71	65.0	3.43	7.16	10.59	17.73	7.14	40.3	0.84
28	125	50	3.13	0.12	3.25	14.93	11.68	78.2	2.05	7.57	9.62	17.73	8.11	45.8	0.84
29	129	50	2.85	0.09	2.94	14.93	11.99	80.3	1.93	6.54	8.47	17.73	9.26	52.2	0.84
30	126	50	2.80	0.10	2.90	14.93	12.03	80.6	1.79	6.34	8.13	17.73	9.60	54.1	0.84
31	131	50	4.51	0.11	4.62	14.93	10.31	69.1	3.24	6.90	10.14	17.73	7.59	42.8	0.84
Nov.															
1	131	50	6.57	0.11	6.68	14.93	8.25	55.2	4.45	7.31	11.76	17.73	5.97	33.7	0.84
2	134	50	2.34	0.14	2.48	14.93	12.45	83.4	1.71	8.45	10.16	17.73	7.57	42.7	0.84
3	131	50	2.07	0.20	2.27	14.93	12.66	84.8	1.30	6.10	7.40	17.73	10.33	58.3	0.84
4	132	50	1.25	0.14	1.39	14.93	13.54	90.6	1.77	6.66	8.43	17.73	9.30	52.5	0.84
5	136	50	2.69	0.26	2.95	14.93	11.98	80.2	1.54	6.63	8.17	17.73	9.56	53.9	0.84
Average			3.58	0.13	3.71	14.93	11.22	75.1	2.44	6.91	9.35	17.73	8.38	47.3	0.84

Calcium and Phosphorus Balances

The results of the metabolism study are summarized in table 2. The balances for both animals are in fairly close agreement. Animal V3 developed severe diarrhea soon after the addition of viosterol to the ration which continued until the fatal termination. During the last three days the feces had a putrid odor, contained fat and occasionally small amounts of blood. V3 also manifested a lack of appetite soon after the viosterol was fed. In spite of this condition the animal made good use of both calcium and phosphorus. At autopsy V3 showed marked hemorrhagic gastritis, the true stomach contained a bloody curd and the rumen was filled with coagulated milk, $CaH_4(PO_4)_2 \cdot H_2O$ and viosterol. The kidneys were soft, enlarged and covered with large reddish gray spots. No abnormal calcification was visible by macroscopic examination. The bile sac in V3 was entirely empty.

Animal V4 reacted somewhat differently to the heavy feeding of viosterol. The appetite remained fairly good, the animal looked and acted normal and gained in weight. The viosterol feeding was continued for sixteen days, after which time the animal was killed and a post-mortem examination was made. During the last few days of the metabolism the feces became very soft or liquid, contained some fat and occasionally small blood clots. At autopsy, V4 showed the same general condition as V3 but, in addition, the lymph nodes along the backbone were usually large. The bile sac in V4 contained a small amount of bile, the analysis of which is given in table 4.

In general, the results of this metabolism show that the feeding of excessive doses of viosterol caused a decrease in the excretion of fecal calcium and phosphorus and markedly increased the excretion of urinary calcium under the conditions of this experiment. The animals remained in a high positive calcium and phosphorus balance because of the adequate food intake. The absorption of calcium and phosphorus from the intestinal tract was greater during the viosterol feeding period than during the fore-period. It is unfortunate that balances were not determined for nitrogen. Toward the end of the experiment, large albuminous precipitates formed in the urine indicating extensive renal damage. This observation is in agreement with Spies and Glover (16).

Some of the soft tissues were analyzed for calcium, phosphorus and magnesium to determine the relative amounts of these elements present. These results are presented in table 3.

The analyses of the various tissues of young normal calves are not available. However, Hess, Benjamin and Gross (17) and others have shown that calcium and phosphorus are present in these tissues in dogs in larger amounts than normal.

TABLE 3

Calcium, phosphorus and magnesium content of organs of calves fed large amounts of viosterol

	CALF V3			CALF V4		
	Ca	P	Mg	Ca	P	Mg
	Per 100 gm. dry tissue			Per 100 gm. dry tissue		
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Liver	50.8	1023.0	82.0	37.0	1160.4	91.6
Kidney	250.0	1200.5	86.2	140.0	1169.0	97.4
Heart	128.3	857.8	87.2	27.1	477.8	57.5
Lymph-nodes	.....	.....	.....	53.3	928.5	66.1
Aorta	.....	.....	.....	301.7	390.0	19.8

The ash content of the right dental pad of V4 was found to be 55.8 per cent. The percentages of bone ash of these three calves are in agreement with values we have for normal animals. The ash content of the dental pads of rachitic calves show a much lower value (18). It is unlikely that any unusual amount of calcium or phosphorus was removed from the bones of the calves due to high viosterol feeding, because of the short time they were on experiment.

TABLE 4

Total milligrams of calcium, phosphorus and magnesium in the biles

	BILE	Ca	P	Mg
	gm.	*mg.*	*mg.*	*gm.*
V1	31.0	15.85	34.56	3.63
V2	46.0	12.19	41.16	3.59
V3	none	.....	.....	.....
V4	7.64	1.56	5.95	0.59

The grams of bile and the milligrams of calcium, phosphorus and magnesium in the bile of the three calves are given in table 4. V1 and V2 had about the normal quantity of bile for a young calf but the color and viscosity were abnormal. V3 and V4, which received the higher dosages of viosterol, had either no bile in the sac or only a small quantity of abnormal bile. The bile in all of the animals was characterized by a yellowish-brown color and high viscosity. These observations are of interest in view of the fact that the bile sacs of rachitic calves are unusually large and contain from 100 to 800 cc. of yellow to brick-red colored bile.

SUMMARY

The feeding of large daily doses of viosterol to young calves decreased the total excretions of calcium and phosphorus.

There was an increase in the absorption of calcium and phosphorus from the intestine. The excretion of calcium in the feces was decreased, whereas the excretion in the urine was greatly increased.

The concentration of calcium in the blood plasma was not as markedly increased as the inorganic phosphorus due to viosterol feeding. There was a tendency for the blood calcium to decrease and the phosphorus to increase some hours prior to death.

Calves apparently show an idiosyncrasy to the development of hypercalcemia under the conditions of this experiment.

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PEDIGREE ANALYSIS AS A BASIS OF SELECTING BULL CALVES

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Proved sires have been advocated much during recent years as the soundest means of herd improvement. In view of the evidence advanced by critical research and the results of practical dairy experience, there seems to be little argument about this point. Yet, judging from progress now being made, it will be many years before any appreciable percentage of dairy calves are sired by good proved bulls.

For instance, the New York State Dairy Herd Improvement Association report for 1932 shows a total of 34,281 cows tested, yet only 134 bulls proved. Of this number only 25 were alive when proved. In any event, it appears that a great number of untried young bulls must continue to be used. Every proved sire was once an untried calf and some breeder had to take a chance in proving him. It is a tragedy and a severe loss to the industry that so many of these prospective herd improvers are failures when proved. Even when a young bull does prove out and effects an improvement in the herd, he is too often followed by another young sire that immediately reduces production back to its original low level and the actual improvement to the breed is negligible.

It is a serious challenge to constructive breeders and to students of breeding to uncover additional facts and supply information to dairymen that will materially assist them in the selection of untried bull calves, calves that will in the majority of cases prove out successful. Obviously, in the use of any untried young bull an element of chance is involved. The purpose of this study was to ascertain just what available information is needed by a breeder to lessen this element of chance and to be able to select a bull calf with a fair degree of certainty that he will at least maintain and if possible improve production in the herd.

The type of the calf and a study of his ancestry furnish the only basis for such selection. We know that the individual's conformation alone is not a reliable guide. Too many grand champion winners have developed into failures as sires of production for a great deal of dependence to be placed on this method. Consequently, the ancestry of the calf as shown by the pedigree remains the important basis for study.

In June of 1932 the American Jersey Cattle Club adopted a new classification for bulls, called "Tested Sires." To qualify as a "Tested Sire" it is required that a Jersey bull must have at least ten officially tested daughters with lactations of 270 days or more in length. The new classifica-

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tion displaced the old system of Register of Merit Sires which had been in operation for a number of years. This action was taken by the American Jersey Cattle Club as a result of extensive studies made by Gifford (1) and Gowen (2) and confirmed by research in the Club office (3). These studies indicated that the average production of a bull's first ten tested daughters furnishes a very good estimate of what his future progeny will produce. Edwards (4) in recently studying this problem by means of a different method of attack arrives at substantially the same conclusions.

The first volume of these "Tested Sires" was published in January 1933. This volume contains the names of 729 bulls and gives for each sire the total number of tested daughters and the average yield of all tested daughters computed to maturity. The present study is based on the data published in this book.

The plan of the investigation was to tabulate and examine carefully the performance of the ancestry of all the bulls listed in the "Tested Sire" volume and from such a study ascertain the possible reasons that might explain why some of these bulls sired exceptionally high producing daughters while others transmitted only a low level of production.

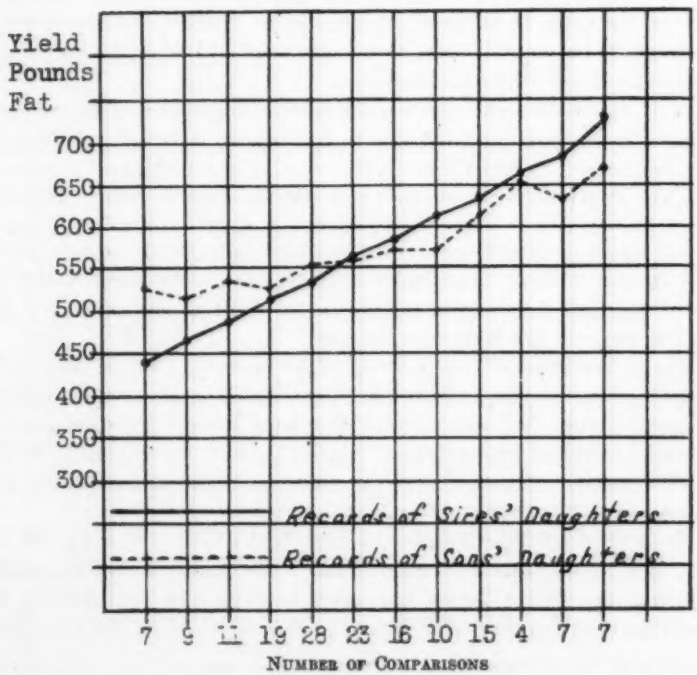

Fig. 1. Comparison of records of the daughters of "Tested Sires" with the records of their sons' daughters.

There are 156 bulls with ten or more tested daughters that in turn have sons with ten or more tested daughters. The relationship or correlation between the records of the sires' daughters and the records of the sons' daughters should furnish an indication of the value of the records of the sires' daughters in measuring the transmitting ability of the son. The results of this comparison are portrayed in Table 1 and Figure 1.

Of the 729 sires, 385 were out of tested dams. Again, the relationship between the dams' records and the sons' daughters' production will indicate the value of a production record in evaluating the transmitting ability of the dam. In other words, the records of these 385 dams were compared with the average of the records of their sons' daughters. The results of this comparison are shown in Table 1 and Figure 2.

Examination of this table and the accompanying figures reveals that the coefficient of correlation between the records of the sires' daughters and the records of the sons' daughters is very good, while the correlation between the dams' own records and the sons' daughters' records is only fair. These results are significant and demonstrate that in the selection of a bull calf it is more important to consider the average production of his sire's daugh-

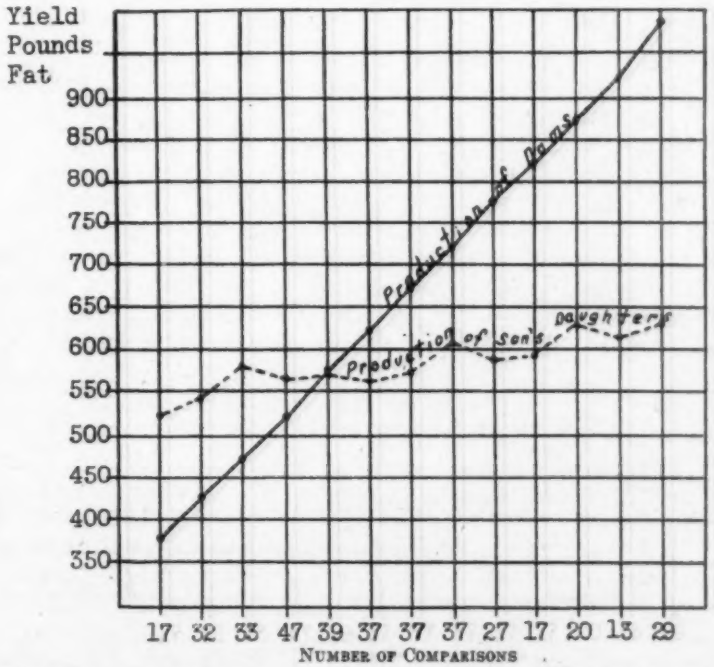

Fig. 2. Comparison between records of dams and records of their sons' daughters.

ters than it is to limit consideration to his dam's record alone. The correlation between the dams' records and sons' daughters' records compares favorably with that previously reported by the author (5). Gowen (6) working with Holsteins found a slightly less correlation ($+0.307 \pm 0.047$) between maternal grandams and granddaughters. It is to be noted that the genotypes of the bulls have been compared to the genotypes of their sires but to the phenotypes of their dams.

TABLE 1

Physical constants for sires' and sons' daughters and dams and sons' daughters

	MEAN	STANDARD DEVIATION	CORRELATION BETWEEN SIRES' DAUGHTERS AND AND SONS' DAUGHTERS	CORRELATION BETWEEN DAMS' RECORDS AND SONS' DAUGHTERS
Sires	565.16 ± 3.9	72.14 ± 2.7	$+0.558 \pm .055$	
Sons' daughters	572.21 ± 3.6	65.74 ± 2.5		
Dams	651.72 ± 5.9	172.61 ± 4.1		$+0.331 \pm .031$
Sons' daughters	581.88 ± 2.8	79.92 ± 1.9		

The author's attention has been called to the possibility of the range in production of a bull's daughters furnishing valuable information in regard to his transmitting ability. In studying the records made by the progeny of a bull, is it important to know what proportion of his daughters are above the breed average and what proportion are inferior? To study this phase of the question the group of 156 sires having "Tested" sons was used. The daughters of each sire were segregated in various groups, depending on their butterfat yield. Then the percentages of daughters over 500 pounds of fat, over 550 pounds, over 600 pounds and over 650 pounds were determined for each bull. These percentages were next compared with the average yield of the sons' daughters. The results are given in Table 2. It will be observed that the percentage of a bull's daughters over 600 pounds of fat is just about equal to but not any better than the average production of all his tested daughters in estimating the production of the sons' daughters.

There were 312 sires with ten or more tested daughters that in turn had paternal grandsires with ten or more tested daughters. The average yields of the paternal grandsires' daughters were compared with the average yields of the grandsons' daughters. This was done in order to estimate the amount of consideration that should be given to the paternal grandsire in pedigree study. Similarly, it was found that 241 of the bulls listed had maternal grandsires with ten or more tested daughters. A like comparison was made between the average yield of the maternal grandsires' daughters

TABLE 2

Correlation coefficients of ranking of sires' daughters and yield of sons' daughters

	MEAN	CORRELATION BETWEEN PERCENTAGES OF SIRES' DAUGHTERS OVER 500 LBS., OVER 550, 600 AND 650 LBS. AND YIELD OF SONS' DAUGHTERS
Percentage of sires' daughters over 500 lbs.	64.3 ± 1.25	+0.434 ± .044
Percentage of sires' daughters over 550 lbs.	48.9 ± 1.28	+0.496 ± .041
Percentage of sires' daughters over 600 lbs.	35.1 ± 1.24	+0.550 ± .038
Percentage of sires' daughters over 650 lbs.	24.7 ± 1.11	+0.528 ± .038
Yield of sons' daughters	572.2 ± 3.6	

and the grandsons' daughters. The results of these comparisons given in Table 3 show a significant difference between the correlation figures to the two grandsires.

TABLE 3

Physical constants for paternal and maternal grandsires' daughters and grandsons' daughters

	MEAN	STANDARD DEVIATION	CORRELATION BETWEEN GRANDSIRES' DAUGHTERS AND GRANDSONS' DAUGHTERS
Paternal grandsires' daughters	534.05 ± 2.3	60.45 ± 1.6	+0.250 ± .036
Grandsons' daughters	576.52 ± 2.7	71.76 ± 1.9	
Maternal grandsires' daughters	554.18 ± 3.2	72.72 ± 2.2	+0.427 ± .036
Grandsons' daughters	577.54 ± 3.3	75.60 ± 2.3	

The possibility that some of the factors governing the inheritance of milk yield may be inherited in a sex-linked manner has been suggested previously by Smith, Scott & Fowler (7) and Madsen (8). Gowen (9), however, believes that the sire and the dam are equally and jointly responsible for the milk production and butterfat percentages of their offspring, also Fohrman and Graves (10) doubt the assumption of sex-linkage in the inheritance of milk yield.

An interesting side-light on this phase of the problem was accidentally encountered in looking up information on the bull, Pogis 99th of Hood Farm. A pronounced difference was observed in the production of the

grandsons' daughters where Pogis 99th of Hood Farm occurred as the maternal grandsire instead of the paternal grandsire. In checking through the "Tested Sire" booklet it was found that of the bulls whose daughters averaged over 600 pounds of fat there were just three that had five or more "tested" grandsons sired by sons and five or more "tested" grandsons out of daughters. The names of these three bulls are Pogis 99th of Hood Farm 94502, Fauvic's Prince 107961 and Hood Farm Torono 60326. The data on these sires is shown in table 4.

In all three of these examples it would appear that where the bulls named occurred as the maternal grandsires they transmitted the factors of production on to their grandsons to a slightly greater extent than they did when they appeared as the paternal grandsires.

TABLE 4

NAME OF GRANDSIRE	NO. OF DAUGHTERS	AVER. YIELD OF DAUGHTERS	NO. OF PATERNAL GRANDSONS	AVER. YIELD OF DAUGHTERS OF PATERNAL GRANDSONS	NO. OF MATERNAL GRANDSONS	AVER. YIELD OF DAUGHTERS OF MATERNAL GRANDSONS
P 99 of H. F.	119	693.88	12	580.86	10	619.04
F. P.	62	728.08	5	557.52	5	604.22
H. F. T.	72	637.34	14	581.03	22	600.57

These results might be explained as indicating a sex-linked inheritance and it would appear not unreasonable to assume that at least a few of the factors affecting the inheritance of milk production may be transmitted on the sex chromosome. On the other hand it will be noted that the dams of the tested sires were a highly selected group having an average butterfat yield considerably above the breed average. Furthermore, as shown further on, the daughters of these dams are considerably above the breed average in producing ability. In other words, it is possible that the daughters of the maternal grandsires were a more highly selected group than were the sons of the paternal grandsires. It may be that this explanation will account for all of the difference between the correlation to the paternal grandsires' daughters and the maternal grandsires' daughters. It is to be hoped that further work will be undertaken in connection with the other breeds to present further evidence on this important question.

Through the progeny test we have a very good means of predicting a bull's potential transmitting ability. Similarly, the best index of the germinal composition of the dam would be her transmitting ability of desired characters to her daughters. This index is not available to the same extent that it is in the case of bulls. Very few cows have as many as even 5 tested daughters. It was found that there were 51 bulls listed that were out of

cows having three or more tested daughters. The records of the daughters of these dams were averaged and the averages of their production were compared to the average productions of the sons' daughters. Ninety bulls listed were out of dams having two tested daughters and 172 of the bulls were out of dams having one tested daughter. Similar comparisons were made between the averages of these dams' daughters and the averages of the sons' daughters. The results are pictured in table 4. Where the dam has three or more tested daughters, the amount of correlation is particularly good. In fact the record of only one tested daughter seems to be equal to the dam's own record in rating her transmitting ability.

TABLE 5

Coefficients of correlation of dams' daughters and sons' daughters

	MEAN	STANDARD DEVIATION	CORRELATION BETWEEN DAMS' DAUGHTERS AND SONS' DAUGHTERS
Dams with three or more tested daughters	615.61 ± 10.50	111.16 ± 7.43	+ 0.466 ± .074
Sons' daughters	593.67 ± 7.45	78.87 ± 5.27	
Dams with two tested daughters	574.44 ± 6.07	85.45 ± 4.30	+ 0.378 ± .061
Sons' daughters	594.58 ± 5.56	78.27 ± 3.94	
Dams with one tested daughter	600.06 ± 7.76	150.63 ± 5.49	+ 0.357 ± .045
Sons' daughters	581.86 ± 3.99	77.44 ± 2.82	

The average production of all (9265) 365 day Register of Merit records ever completed by mature cows from 5 to 10 years of age, inclusive, is 549.22 pounds of butterfat. This, it appears, may be reasonably considered as the mature average 365 day production of the breed for cows tested in the Register of Merit. The average production of the daughters of the 729 "Tested" sires computed to maturity is 557.71 pounds of fat. Thus the daughters of the 729 sires listed were slightly better than the breed average although in the list there were 162 bulls whose daughters averaged less than 500 pounds of fat and of these 42 had daughters averaging less than 450 pounds. Such bulls are apparently heterozygous in their genetic make-up. On the other hand, the germinal composition of the bulls whose daughters average over 600 pounds of fat should be quite homozygous. If this is so, an examination of the transmitting ability of such sires should give valuable results.

Of the 156 bulls with "Tested" sons, the daughters of only 43 average above 600 pounds of fat. Taking these 43 sires for study, it was found that 25 (58.14%) had sons whose daughters in turn average above 600 pounds of fat. Of the 385 cows with "Tested" sons, 217 have records higher than 600 pounds of butterfat. The sons of 95 (43.78%) of these cows in turn sired daughters averaging above 600 pounds of fat.

There were 42 cases in which the daughters of paternal grandsires of "Tested" sires averaged over 600 pounds of fat. Twenty (47.62%) of the grandsons of these 42 bulls in turn sired daughters averaging over 600 pounds. Examination of the list of "Tested" sires having "Tested" maternal grandsires revealed that there were 59 of such maternal grandsires whose daughters average over 600 pounds of fat. Thirty-four (57.63%) of their grandsons sired daughters averaging over 600 pounds of fat. Grouping the dams with three or more tested daughters and those with two tested daughters together gave a total of 54 dams with two or more tested daughters averaging over 600 pounds of fat. Thirty-

TABLE 6

Tabulation of sires with high producing and high transmitting ancestry.

	TOTAL NUMBER	NUMBER OVER 600 LBS. FAT	NUMBER OF SONS OR GRANDSONS WITH DAUGHTERS AV. 600 LB. FAT	PERCENTAGE OF SONS OR GRANDSONS WITH DAUGHTERS AVERAGING OVER 600 LBS. FAT
"Tested" bulls with "Tested" sons	156	43	25	58.14%
Record cows with "Tested" sons	385	217	95	43.78%
"Tested" paternal grandsires with "Tested" grandsons	312	42	20	47.62%
"Tested" maternal grandsires with tested grandsons	241	59	34	57.63%
Dams with 2 or more record daughters and having "Tested" sons	141	54	31	57.41%
"Tested" bulls by "Tested" sires whose daughters av. over 600 lbs. and whose dams have 2 or more daughters averaging over 600 lbs.		27	21	77.78%
"Tested" bulls by "Tested" sires whose daughters av. over 600 lbs., whose dams have 2 or more daughters averaging over 600 lbs. and whose maternal grandsires 10 or more record daughters av. 600 lbs.		14	12	85.71%

one (57.41%) of the sons of these dams sired progeny averaging over 600 pounds of fat. These results compare favorably with the correlation data and indicate that in studying a pedigree more weight should be given to the sire's daughters, dam's daughters and maternal grandsire's daughters than to the dam's record or the paternal grandsire's daughters. Another reason that the paternal grandsire's daughters need not be given so much importance is that the records of the sire's daughters represent the inheritance from the sire's side.

"Tested" sires out of dams having two or more daughters averaging over

Following on this line of work, it was found that there were 27 600 pounds of butterfat and in turn sired by bulls whose daughters all average about 600 pounds of fat. Twenty-one, or 77.78% of these bulls sired daughters, the average of whose production exceeded 600 pounds of fat. Going back still further in the pedigree, it was ascertained that there were just 14 bulls whose sires' daughters, dams' daughters and maternal grandsires' daughters all average over 600 pounds of fat. Twelve of these bulls, or 85.71%, in turn sired daughters averaging over 600 pounds of fat and in the other two instances the daughters averaged considerably over 500 pounds of butterfat. The accompanying Table 5 illustrates these results.

SUMMARY AND CONCLUSIONS

1. In studying the ancestry of a bull calf the records of the sire's daughters are considerably more valuable than is the record of the dam alone.

2. On the evidence submitted, the records of the daughters of the maternal grandsire are more closely related to the production of the grandsons' daughters than are the records of the daughters of the paternal grandsire.

3. It would appear that if a cow has a production record herself and if she has two or more tested daughters and if in turn her sire has a number of tested daughters, the sum of this information gives a good index of her germinal composition. This combined information is necessary to accurately evaluate a cow's transmitting ability.

4. The selection of a calf with a pedigree as follows should reduce to a minimum the element of chance involved. A large majority of young bulls with such pedigrees should sire high producing daughters, particularly if bred to cows possessing an average inheritance of production.

Prospective bull calf	Sire	Ten or more tested daughters averaging over 600 lbs. of fat.*	Paternal Grandsire If possible 10 or more tested daughters averaging over 600 lbs. although not absolutely essential. Paternal Grandam
	Dam	Individual record over 600 lbs.* Two or more tested daughters averaging over 600 lbs.*	Maternal Grandsire If possible 10 or more tested daughters averaging over 600 lbs. of fat.* Maternal Grandam

* All records computed to a mature 365 day basis.

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THE MANUFACTURE OF LOW-LACTOSE SKIM MILK FOR USE IN ICE CREAM

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The percentage of milk-solids-not-fat ordinarily used in ice cream mixes has been limited by the amount of lactose which could be incorporated in a mix without any great possibility of the development of sandiness. A method by which the percentage of milk proteins could be increased without increasing the percentage of lactose in the mix beyond that which causes sandiness, would be of value in improving the body and texture of ice cream, in safeguarding the product against possible crystallization of the lactose, and in increasing the consumption of milk solids.

One patented method (1) of increasing the milk solids of an ice cream mix consists in the addition of milk powder to the mix during freezing, but such a procedure also incorporates sufficient lactose to produce a supersaturated solution of this sugar in cases where the frozen product is partially melted or subjected to heat-shocking and subsequent freezing. Another method involves the addition of any one of several soluble milk proteins found on the market. These proteins are not in the state in which they normally occur in skim milk; they have been removed by chemical means and redispersed, in most cases in weakly-alkaline solutions.

The experimental work reported in this paper was undertaken with the object of perfecting a method for the removal of a large percentage of the lactose from skim milk without changing the normal dispersion of its proteins. Experiments showing the advantages of this low-lactose skim milk (for convenience this product is called "low-lac") in ice cream have been carried out.

The basic data, upon which the process for manufacturing this low-lactose milk rests, have been reported by Leighton and Leviton of these laboratories (2). These investigators have shown "that the addition of cane sugar in suitable amounts to skim milk before condensing markedly lowers the viscosity of the highly concentrated product," and that "this phenomenon can be attributed to the diluting action of the cane sugar on the basis that the dissolved sugar markedly increases the volume of the liquid phase of the suspension." Leighton and Leviton have also shown that the cane sugar inhibits an increase in the viscosity of the concentrated milk on standing, and furthermore that, by means of this addition of cane sugar to skim milk before condensing, the viscosity of the concentrated product is reduced "to the point where lactose crystallization can take

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place readily and where separation of the milk sugar crystals by mechanical means is feasible."

MANUFACTURE OF LOW-LAC MILK

The optimum quantity of cane sugar to add to 100 pounds of skim milk for maintenance of minimum viscosity in the concentrated product has been shown by Leighton and Leviton to be 5.9 pounds. The mixture should be forewarmed to 65° C. for 10 minutes before it is drawn into the vacuum pan, where a concentration of 68 to 70 per cent total solids is attained. As an aid in striking the batch a striking cup may be used. The specific gravity of the finished milk at 40° C. should be between 1.32 and 1.34, and its viscosity at this temperature will range between 26 and 32 centipoises (2). The refractive index of the condensed product at 40° C. should be approximately 1.470 to 1.490. After it is drawn from the pan the concentrated milk is next cooled to between 20° and 25° C. in cold water with occasional slow stirring. It is then held about 20 hours in an ice box at 10° C.; this temperature being preferred to a higher temperature (where maximum crystallization would occur) in order to prevent the development of excessive viscosity, a characteristic of milks held at high storage temperatures. During this time the lactose crystallizes and can be removed from the milk by means of a basket centrifuge, or preferably by means of a filter press.

The separation of the lactose crystals is the only step in the process which offers any serious obstacle to the preparation of low-lac by any dairy plant equipped with a vacuum pan. The milk sugar forms small crystals, the removal of which from the heavy condensed milk renders special equipment necessary. When the viscosity of the product is not excessively great, filter pressing is the most rapid means of separating the lactose. For all viscosity conditions and crystal sizes, centrifugal separation is very satisfactory. For either centrifuge or filter press the filter cloths which have been found satisfactory are of very fine mesh.

The quantity of lactose which can be removed from the condensed milk will vary from 40 to 75 per cent of the total lactose present in the milk. Under favorable conditions an average of 60 to 70 per cent can be removed.

There is less tendency toward bacterial spoilage in low-lac than in plain condensed skim milk because of the added cane sugar. The parts of cane sugar to 100 parts of water (sugar ratio) will vary in low-lac from 45 to 50 which is considerably below the required figure of 63 to 65 necessary to prevent bacterial growth. Additional cane sugar may be added but a very heavy bodied milk will result.

The viscosity of low-lac increases during storage, a high viscosity developing more rapidly where the temperature of storage is high. For routine production it may be found desirable to dilute the finished product with plain skim milk to attain a uniform concentration of about 50 per cent total

solids. By thinning the milk in this manner the inconvenience of handling a heavy bodied product will be avoided.

Approximately 50 different batches of low-lac have been made in these laboratories, using about 200 pounds of skim milk per batch. The method of manufacture has also been found satisfactory when tried out on a semi-commercial scale at the Grove City Creamery, Grove City, Pa., where batches of approximately 1,400 pounds of skim milk were converted into low-lac.

The cost of manufacture of low-lac above that of normal condensed milk lies only in the labor necessary to remove the lactose. The additional labor consists of two steps: (1) Dropping the condensed milk from the condenser into crystallizing cans where it can be cooled and held over night, and (2) separating the crystals from the milk. The lactose thus obtained may be washed and marketed as crude lactose, or it may be recrystallized to prepare a relatively pure sugar. The expense for the above operations will vary with local conditions but it seems probable that the market value of the lactose would, under normal circumstances, cover the cost of separating it. It should be possible to obtain from 2 to 2½ pounds of washed crude lactose per 100 pounds of skim milk.

In table 1 the data from a number of representative batches of milk are reproduced. Determinations of total solids were made on each condensed milk before and after removal of the lactose. From these figures the percentage of lactose removed was calculated.

TABLE 1

Showing the total solids of representative batches of skim milk with the calculated percentage of lactose removed

BATCH NO.	TOTAL SOLIDS		LACTOSE REMOVED
	Condensed, after evaporation	Low-lac after removal of lactose	
	per cent	*per cent*	*per cent*
1	67.70	63.12	57.8
2	71.42	66.06	69.8
3	67.10	62.91	52.8
4	71.42	66.70	62.1
5	71.09	65.33	74.4
6	70.74	64.40	79.0
7	71.18	67.12	54.6
8	69.31	63.41	72.7
9	74.91	69.99	67.8
		Average percentage lactose removed	65.6

CALCULATIONS INVOLVED IN THE MANUFACTURE AND USE OF LOW-LAC

The calculations necessary to properly control the composition of an ice cream mix when low-lac is used are somewhat involved. Removal of part

of the lactose from the milk alters the ratio of lactose to other milk solids. This altered ratio together with the percentage of lactose desired in a mix must be considered in calculating the quantities of the other ingredients. The percentage of lactose separated from the skim milk is easily calculated using the following formula:

$$1-\frac{f(100-c)}{c(100-f)}\times 100 \quad k = \%\ \text{lactose removed}$$

where: f = per cent total solids of low-lac filtrate
c = per cent total solids of condensed skim milk
k = a constant which changes only with the composition of fresh skim milk

For accurate results the composition of the fresh skim milk should be ascertained by analysis, but for the purpose of this work satisfactory figures were secured by considering the average composition of skim milk to be (3) as follows:

Water	90.35%
Protein	3.27%
Fat	0.15%
Lactose	4.98%
Ash	0.80%

Using these figures together with the quantities of fresh skim milk and cane sugar used in the batch the components of k become:

.0498 = lactose in 1 part fresh skim milk
.0965 = total solids in 1 part skim milk
160 = lbs. milk used
9.5 = lbs. sugar used

and $$k=\frac{(.0965\times 160)+9.5}{.0498\times 160}=3.13$$

Assuming a batch of low-lac is made, and that the condensed skim milk before removal of the lactose tested 71.4 per cent total solids and the low-lac tests 66.0 per cent total solids then:

$$\text{Percentage lactose removed} = 1-\frac{66.0(100-71.4)}{71.4(100-66.0)}\times 313 = 69.79\%$$

It is necessary to know the composition of the low-lac in order to be able to calculate the ice cream mixes. The following example illustrates the method used to obtain these figures.

Since 9.5 pounds cane sugar was added to 160 pounds skim milk, the total weight of the material before condensing was 169.5 pounds. The percentage of cane sugar was then $\frac{9.5}{169.5}\times 100 = 5.60$. Percentages of the

various constituents in the condensed milk may be found by substitution in the proportion:

Percentage total solids in sweetened skim : percentage total solids in condensed : : percentage *a* in sweetened skim : percentage *a* in condensed, or

$$\frac{100}{105.6}(9.68)+5.60 : 71.4 : : \frac{100}{105.6}(\%\ a \text{ in skim}) : \%\ a \text{ in condensed}$$

From this, $4.58 \times \%\ a$ in skim $= \%\ a$ in condensed, except for the cane sugar, for which $4.84 \times \%$ cane sugar in sweetened skim $= \%$ cane sugar in condensed.

Whence, in the condensed:

Protein plus ash	20.84%
Lactose	22.81%
Cane sugar	27.09%
Fat	0.69%
Total solids	71.43%

Of the lactose in the condensed, 69.8% of 22.81, or 15.92, is the proportion removed, 6.89 remaining. The low-lac is then $100 - 15.92 = 84.08\%$ of the condensed. Hence the composition of the low-lac is the following:

$$\frac{20.84}{0.8408} = 24.78\% \text{ protein plus ash}$$

$$\frac{6.89}{0.8408} = 8.20\% \text{ lactose}$$

$$\frac{27.09}{0.8408} = 32.22\% \text{ cane sugar}$$

$$\frac{0.69}{0.8408} = \frac{0.82\% \text{ fat}}{66.02\% \text{ total solids}}$$

Low-lac should be used in an ice cream mix only when there is likelihood that the lactose of normal skim milk, upon further addition of this product, may cause sandiness. Low-lac is intended only to supplement the milk products which normally supply the serum solids of a mix. For example, a mix of 10 per cent milk-solids-not-fat should be made without low-lac. However, a mix of 12 per cent milk-solids-not-fat should derive about 9 per cent of its milk solids from the usual sources (cream, and skim milk) and 3 per cent from low-lac.

There are several methods by which ice cream mixes can be calculated when low-lactose milk is to be used. The method given below appears to be the most simple since it makes use of the familiar square method of standardization. It should here be borne in mind that although an appreciable amount of calculation may be necessary in the manufacture and use of the

first few batches of low-lac, considerable standardization can be worked out which will eliminate most of the calculations.

In calculating a mix it is necessary first to ascertain the percentage of milk-solids-not-fat to be derived from products having a normal lactose content such as skim milk and cream and also that percentage of solids-not-fat to be added in the form of low-lac. Table 2 gives the percentage of lactose which will normally be present in mixes of the designated milk-solids-not-fat percentages. A mix in which the lactose exceeds 5.24 to 5.50 per cent, corresponding to a milk-solids-not-fat content of 10.0 to 10.5 per cent will generally be susceptible to the development of sandiness.

TABLE 2

Showing the percentage of lactose in mixes having a normal ratio of M.S.N.F. to lactose

$$(\%\ \text{Lactose} = \frac{\text{M.S.N.F.}}{1.908}$$ when lactose is present in the ratio of 4.98 per cent to 9.50 per cent milk-solids not-fat)

% M.S.N.F.	% Lactose
10.0	5.24
10.5	5.50
11.0	5.77
11.5	6.03
12.0	6.29
12.5	6.55
13.0	6.82

As an example of the method of calculation consider the composition of the low-lactose milk and of the ice cream mix to be as follows:

Milk constituent	Desired mix composition %	Composition of low-lac %
Fat	12.0	.82
M.S.N.F.	12.0	32.98
Cane sugar	14.0	32.22
Gelatin	0.3	
T.S.	38.3	66.02

Let the limiting lactose content of the mix be 5.24 per cent. With a normal source of milk solids a mix of 12.0 per cent S.N.F. would contain 6.29 per cent lactose.

If the entire 12.0 per cent of solids-not-fat was made up of this low-lac then in the following proportion x = the percentage which would be lactose:

$$32.98 : 8.20 : : 12 : x$$

$$x = 2.99$$

Now the familiar standardization square may be used to ascertain the proportion of lactose which is to be obtained from normal milk solids and from the lactose-free skim milk.

2.99 ——— 1.05 = parts lactose from low lac

desired % lactose 5.24

2.25 = parts lactose from normal milk

6.29 ——— 3.30 = total parts

Converting parts lactose to per cent milk-solids-not-fat:

$\frac{2.25}{3.30} \times 12 = 8.18$ % S.N.F. to be obtained from normal condensed milk and cream.

$\frac{1.05}{3.30} \times 12 = 3.82$ % S.N.F. to be obtained from low-lac

Therefore:

$$\frac{\%\ \text{S.N.F. from low-lac}}{\%\ \text{S.N.F. in low-lac}} \times \text{lbs. mix desired} = \text{lbs. low-lac to be used}$$

$$\frac{3.82}{32.98} \times 100 = 11.58 \text{ lbs. low-lac for 100 lbs. of this mix}$$

With this quantity of low-lac there will also be added

$$11.58 \times 32.22 \div 100 = 3.73 \text{ lbs. cane sugar}$$
$$11.58 \times \ .82 \div 100 = \ .95 \text{ lbs. fat}$$

For 100 pounds of the original mix there now remain the following weights of ingredients to be secured and added in the usual way:

M.S.N.F.	= 12.00 − 3.82 =	8.18 per cent
Fat	= 12.00 − .95 =	11.05 " "
Sugar	= 14.00 − 3.73 =	10.27 " "
Gelatin		0.30 " "

Calculations, according to the method given above, have been made over a wide range of lactose concentrations and the results obtained are given in table 3. By means of this table the lengthy calculations which it is necessary to make when low-lac is used in a mix may be dispensed with. While the figures given are not strictly accurate they will be found to be sufficiently exact in most cases.

To use table 3 it is first necessary to determine, within one tenth of one per cent, the percentage of total solids both in the condensed milk, before the lactose is removed, and in the resultant low-lac milk. Having obtained these figures subtract the percentage of the total solids in the low-lac milk from the percentage of total solids in the condensed milk. A figure representing this difference will be found in the first vertical column of the table.

TABLE 3

Showing the percentage of lactose removed from condensed skim milk when the percentage of its total solids is lowered over a range of 4.0 to 6.0 and the amount of the resultant low-lac milk to add 100 pounds of ice-cream mix in order to raise the percentage of M.S.N.F. from 10 to 11. (The pounds of cane sugar and of M.S.N.F. in the specified quantity of added low-lac milk are also given)

TOTAL SOLIDS OF CONDENSED MILK MINUS TOTAL SOLIDS OF LOW-LAC MILK		WHEN THE PERCENTAGE OF TOTAL SOLIDS OF THE CONDENSED MILK, BEFORE REMOVAL OF THE LACTOSE WAS—					
		62	64	66	68	70	72
Per cent							
4.0	Lactose removed from condensed milk—per cent	47.5	48.7	49.3	50.6	52.4	53.7
	Low-lac milk to add to ice-cream mix—pounds	10.55	9.96	9.45	8.90	8.21	7.76
	Cane sugar in the added low-lac milk—pounds	2.77	2.72	2.67	2.61	2.50	2.45
	M.S.N.F. in the added low-lac milk—pounds	3.35	3.25	3.18	3.08	2.91	2.83
4.5	Lactose removed from condensed milk—per cent	53.1	53.7	55.3	56.2	57.7	59.9
	Low-lac milk to add to ice-cream mix—pounds	9.28	8.86	8.30	7.87	7.38	6.86
	Cane sugar in the added low-lac milk—pounds	2.47	2.44	2.38	2.34	2.28	2.20
	M.S.N.F. in the added low-lac milk—pounds	2.87	2.83	2.72	2.66	2.56	2.43
5.0	Lactose removed from condensed milk—per cent	58.3	59.3	60.5	61.4	63.3	65.5
	Low-lac milk to add to ice-cream mix—pounds	8.37	7.98	7.46	7.12	6.63	6.16
	Cane sugar in the added low-lac milk—pounds	2.25	2.23	2.17	2.14	2.08	2.01
	M.S.N.F. in the added low-lac milk—pounds	2.52	2.48	2.38	2.34	2.23	2.12
5.5	Lactose removed from condensed milk—per cent	63.3	64.3	65.6	67.1	68.8	70.8
	Low-lac milk to add to ice-cream mix—pounds	7.64	7.25	6.82	6.43	6.00	5.62
	Cane sugar in the added low-lac milk—pounds	2.08	2.05	2.01	1.97	1.91	1.86
	M.S.N.F. in the added low-lac milk—pounds	2.24	2.19	2.12	2.05	1.96	1.88
6.0	Lactose removed from condensed milk—per cent	68.3	69.2	70.8	72.3	73.8	76.1
	Low-lac milk to add to ice-cream mix—pounds	6.97	6.67	6.25	5.88	5.52	5.16
	Cane sugar in the added low-lac milk—pounds	1.92	1.91	1.87	1.83	1.78	1.73
	M.S.N.F. in the added low-lac milk—pounds	1.99	1.96	1.89	1.82	1.76	1.67

All calculations are based on the average composition of skim milk as given in Fundamentals of Dairy Science, by Associates of Rogers, as follows: Protein, 3.72 per cent; lactose, 4.98 per cent; and ash, .80 per cent; total solids, 9.50 per cent. The 0.15 per cent fat has been disregarded.

In cases where the actual values do not fall near those in the table interpolation of the figures in the table may be made.

In each square in table 3, corresponding to the various percentages of lactose removed from milks differing in their original concentration, are found four figures. These are: (1) the percentage of lactose which has been removed from the condensed milk (this figure is given as a matter of information only and is not to be used in figuring mix from this table); (2) the pounds of low-lac milk to be added per 100 pounds of mix to raise the percentage of M.S.N.F. from 10 to 11 (10 per cent M.S.N.F. can be safely attained without the use of low-lac); (3) and (4) the pounds of cane sugar and of M.S.N.F. respectively, which are contained in the low-lac added to the mix. The quantity of cane sugar and of M.S.N.F. thus added to the mix must be taken into consideration in making up the rest of the mix.

To raise the percentage of M.S.N.F. from 10 to 11, use the figures direct from the table, but to raise the percentage of M.S.N.F. to 12 or 13 multiply the weights of ingredients given in the table by 2 or 3 as the case may be.

The following example will serve to illustrate how table 3 may be used in making the calculations for 100 pounds of mix containing 11 per cent M.S.N.F.

Suppose your determinations have shown the total solids in the original condensed milk to be 68.0 per cent, and the total solids in the resultant low-lac milk to be 63.0 per cent. The difference is 5.0 per cent.

Find the figure 5 in the first vertical column of the table and read across to the vertical column that represents the 68 per cent total solids in the original condensed milk. It will be found that 7.12 pounds of low-lac milk should be added per 100 pounds of mix to raise the percentage of M.S.N.F. from 10 to 11.

Also, it will be found that the 7.12 pounds of low-lac milk contains 2.14 pounds of cane sugar and 2.34 pounds of M.S.N.F.

Therefore, in making up a mix that is to contain 14 pounds of cane sugar it will be necessary to add 14 – 2.14, or 11.86 pounds of additional sugar. Similarly, if the mix is to contain 12 pounds of M.S.N.F., there must be added 12 – 2.34, or 9.66 pounds of additional M.S.N.F., which may be obtained from other sources such as condensed milk, skim milk, milk powder, or cream.

Should the values for total solids not fall within 0.1 to 0.2 per cent of those given in the table, a satisfactory approximation may be obtained as follows:

Suppose your determinations have shown the total solids in the original condensed milk to be 68.8 per cent, and the total solids in the resultant low-lac milk to be 64.1 per cent. The difference is 4.7 per cent.

Since there are not constants in table 3 comparable to the 68.8 per cent

TABLE 4

Comparative data on ice-cream mixes made with and without low-lac

MIX NUMBER	FAT	COMPOSITION OF MIX*						FREEZING DATA			PLACINGS BASED ON NUMERICAL SCORES IN ORDER OF PREFERENCE		
		M.S.N.F.			Sugar								
		Source											
		Plain cond.	Low-lac		Total	Cane	Lactose	Freezing time	Maximum overrun	Final overrun	Flavor	Texture	Total
			Cond.	Powder									
	%	%	%	%	%	%	%	Min.	%	%	*Figures refer to mix numbers*		
Expt. No. 1:													
Mix 1	12	12.0	.....	.....	12	14	.....	16	93	93			2-1
Mix 2	12	7.82	4.18	.....	12	14	.....	16	89	89			
Expt. No. 2:													
Mix 1	12	10.0	.....	.....	10	14	5.24	14	86	86			
Mix 2	12	7.67	.....	3.33	11	14	5.24	16	88	84			
Mix 3	12	5.33	.....	6.67	12	14	5.24	21	90	85	1-2-3-4	1-(2-3)-4	1-2-3-4
Mix 4	12	2.7	.....	10.3	13	14	5.24	19	102	82		**	
Expt. No. 3:													
Mix 1	12	10.0	.....	.....	10	14	5.24	20	99	85			
Mix 2	12	7.67	.....	3.33	11	14	5.24	23	113	84			
Mix 3	12	5.33	.....	6.67	12	14	5.24	25	117	85	(1-2)-3-4	4-(2-3)-1	2-3-1-4
Mix 4	12	2.74	.....	10.3	13	14	5.24	27	121	85			
Expt. No. 4:													
Mix 1	12	10.0	.....	.....	10	14	5.55	14	83	83			
Mix 2	12	1.25	10.75	.....	12	14	5.20	16	103	80	1-2-3	2-1-3	2-1-3
Mix 3	12	0.19	13.81	.....	14	14	5.09	13	106	82			
Expt. No. 5:													
Mix 1	12	10.0	.....	.....	10	14	5.55	16	95	80			
Mix 2	10	1.46	10.54	.....	12	14	5.13	16	106	80	(1-2)-3	2-1-3	2-1-3
Mix 3	8	0.71	13.29	.....	14	14	5.64	17	112	82			
Expt. No. 6:													
Mix 1	12	1.2	10.8	.....	12	14	5.17	19	114	81			
Mix 2	12	0.97	11.03	.....	12	16	4.99	16	102	81	1-2-3	2-1-3	2-1-3
Mix 3	12	.....	14	.....	14	14	4.92	14	104	82			
Expt. No. 7:													
Mix 1***	12	12.0	.....	.....	12	14	6.29				1-2	1-2	1-2
Mix 2	12	10.0	2	.....	12	14	5.24						

* All mixes, contained 0.3% gelatin.

** Parentheses indicate tie score.

*** Developed sandiness on heat-shocking after being scored.

and the 4.7 per cent, the rule to follow is to use the nearest higher figure to the 68.0, or 70 in this case, and the nearest lower figure to the 4.7, or 4.5 in this case. Reading across the table from 4.5 to the column headed 70, it will be found that 7.38 pounds of low-lac should be added. The figures for additional sugar and M.S.N.F. are located in the same way.

It will be observed that the more efficient is the separation of lactose from the condensed skim milk, the smaller will be the quantity of low-lac required in the mix. It should also be pointed out that when the sum of the M.S.N.F. added to a mix in the low-lac and in the cream, exceeds the total desired percentage of M.S.N.F., then the upper limit of M.S.N.F. percentage in a given mix has been reached for a low-lac with this particular percentage of lactose removed.

MANUFACTURE OF ICE CREAM FROM LOW-LAC MILK

Ice cream mixes of varying composition were made up using low-lac to increase the milk solids above 10 per cent. Thirty-pound mixes were pasteurized for 30 minutes at 63° C., aged overnight, frozen, hardened and judged by from 10 to 15 persons within a week after freezing. A summary of the data obtained is given in table 4. The numerical scores have been summarized and the results reproduced in the table. Mix No. 1 in each experiment is the control mix for that experiment. It will be observed that in experiments No. 2 and No. 3 low-lac powder was substituted for the low-lac condensed milk. This was an entirely satisfactory spray dried product. However, the control mix in experiment No. 2 was preferred over those which contained low-lac powder. Again in experiment No. 7 the control ice cream was judged preferable over the low-lac product. However, since this latter control contained 12 per cent serum solids, it soon became sandy after slight heat-shocking.

The data in table 4 show that low-lac can be advantageously used to raise the milk-solids-not-fat of ice cream mixes from 10 to 12 per cent. Such ice creams generally show an improved body and texture, a higher total score, and are free from any susceptibility to sandiness. The flavor of the control samples was generally considered equal to or superior to the ice creams containing low-lac. However, the low-lac mixes possessed a firmer, smoother body and texture.

The removal of much of the lactose from skim milk and the subsequent use of this milk in the mix increases the ratio of milk salts to the other milk solids in the product. The effect of this altered ratio is to limit the quantity of milk-solids-not-fat which a low-lac mix may contain to about 13 per cent. This means that if a mix of 13 per cent serum solids is made from a batch of low-lac testing 62.5 per cent total solids and from which 67 per cent lactose has been removed, 6 per cent of the 13 per cent milk-solids-not-fat will be derived from the low-lac and the remaining 7 per cent from

normal sources of supply such as skim milk or cream. It is interesting to note that the proportion of serum solids to be derived from low-lac, when a good separation of lactose has been obtained, is less than half of the total amount in the mix and very often it will not exceed a quarter of the milk-solids-not-fat of the mix.

The low-lac provided a means of successfully substituting milk-solids-not-fat for fat to a limited extent as shown by the data in table 4, experiment 5. The ice cream containing 10 per cent fat and 12 per cent milk-solids-not-fat received a higher score than that containing 12 per cent fat and 10 per cent milk-solids-not-fat.

SUMMARY AND CONCLUSIONS

1. Approximately 65 per cent of the lactose of skim milk has been removed both on a laboratory and on a semi-commercial scale without altering the normal degree of dispersion of the milk proteins.

2. This process consists of the following steps: 5.9 pounds of cane sugar is added to 100 pounds of fresh skim milk, the mixture is forewarmed to 63° C. for 10 minutes, evaporated under vacuum to 70 per cent total solids, drawn and cooled to 25° C., held 20 hours at 10° C., and the crystallized lactose removed from the milk by means of a centrifuge or a filter press.

3. The value of low-lactose milk lies in the opportunity which it provides of increasing the milk-solids-not-fat of an ice cream mix above that amount where lactose crystallization threatens when normal milk solids are used to reach an identical concentration. Ice cream mixes containing 11 to 13 per cent milk-solids-not-fat, in which the concentration of lactose was controlled by the use of low-lac milk, possessed an improved body and texture and were capable of withstanding adverse handling conditions without the development of sandiness.

ACKNOWLEDGMENT

The authors express their appreciation and thanks to W. T. Johnson, Jr., and G. A. Ramsdell for their cooperation and help in applying on a semi-commercial scale, at the Grove City Creamery, this method of lactose separation, and to E. O. Whittier for checking the calculations introduced in this paper.

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FURTHER STUDIES OF THE EFFECT OF PASTEURIZATION ON THE BACTERIAL FLORA OF LOW COUNT MILK

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The absence of acid coagulation and the development of abnormal flavors have been noted frequently in commercially pasteurized milk from different sections of the State of Washington. Similar observations have been made in other states. A recent correspondent (5) of a dairy trade journal made the following statement, which is typical of comments concerning this problem: "I have come to the conclusion that pasteurization equipment is becoming too efficient these days. By this I mean that too many of the lactic acid organisms are being killed and in this condition other organisms thrive which otherwise would have been retarded if a normal number of the lactic type had been left."

Data covering all seasons of the year and including more than 30,000 plate counts compiled from the records of numerous state, county, city, and milk plant laboratories located in various sections of the State of Washington show that approximately 35, 55, and 80 per cent of the market milk of the state reaches the pasteurizing plant with bacterial counts of less than 5,000; 10,000; and 25,000 per cc. respectively.

A study made by Black *et al.* (4) in this laboratory showed that when milk of low count was pasteurized in flasks at 142.5° F. for 30 minutes the proportion of acid-forming bacteria was reduced and that they were not of major importance in the spoilage of the product. Since the above study was made with samples of milk pasteurized in flasks and handled in a manner to prevent subsequent contamination, it seemed advisable to continue this study with the commercially pasteurized product.

LITERATURE REVIEW

Ayers and Johnson (2), from a study of the changes developed in litmus milk by organisms picked from plate cultures, found that pasteurization at 145° F. for 30 minutes caused a marked increase in the relative proportion of acid-forming bacteria to the other types in the milk. They reported similar findings when pasteurization was carried out at both 160° F. and 170° F., but at 180° F. the peptonizing bacteria predominated in the finished product. These findings were obtained on milk of a high initial count, many of the samples containing millions of bacteria per cc.

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The data presented by Ayres and Johnson (2) have been cited in a recent revision of a bulletin originally prepared by Ayers (1) in which it is stated that pasteurized milk sours in a manner similar to that of a high grade raw milk, except that the souring is delayed.

Recently Thurston and Olson (6), using the milk tube technique of Ayers and Johnson (2), found that pasteurization at 145° F. for 30 minutes reduced the percentage of acid-coagulating bacteria and increased the percentage of acid-forming non-coagulating types. The total percentages of these two groups remained unchanged, being 66.18 and 66.86 respectively in the raw and pasteurized milk. They studied the changes in the bacterial flora of six raw and six pasteurized samples held at storage temperatures ranging from 35° to 56° F. The acidities in both the raw and pasteurized samples increased but little during the four day observation period. The pasteurized milks stored at 53° to 56° F. were the only pasteurized samples the acid-forming, the proteolytic, and a group including alkali-forming and in which an appreciable increase occurred in the numbers of organisms. The predominating organisms in the pasteurized samples stored at this temperature after the first day were the inert and alkali-forming types. They found that acid developed slowly in the pasteurized milk and that coagulation occurred at low acidities apparently due to the production of a rennin-like enzyme by the organisms.

PROCEDURE

The data to be presented were obtained from 75 corresponding samples each of raw and pasteurized milk collected during all seasons. All samples represented the mixed milk from several herds. The raw milk samples were collected after the contents of the pasteurizer had been thoroughly mixed and before any temperature increase. Pint bottles of milk from the regular run constituted the pasteurized samples.

Pasteurization was carried out in a Burrell Simplex Spray Pasteurizer, of 200 gallon capacity, at 143° F. to 145° F. for 30 minutes and was followed by immediate cooling and bottling. Steam and hot water, of which there was an abundance, were used in the sterilization of the milk plant equipment.

The bacteria contained within the milk were divided into three groups: inert types. Total and differential bacterial counts were made on the raw and pasteurized samples when fresh and again after 24 hours and 48 hours of incubation at 70° F. This temperature was chosen for the reasons that it is favorable for the development of lactic-acid-forming bacteria and that bottled milk often is subjected to relatively high temperatures for considerable periods before being consumed.

The bacterial counts were determined by the use of Bacto-nutritive caseinate agar, a modification of the medium recommended by Ayers and Mudge

(3). Prior to sterilization 8 cc. of brom cresol purple indicator, prepared by dissolving 0.4 grams of the powdered indicator in 7.4 cc. of N/10 NaOH and 92.6 cc. of distilled water, were added to each liter of medium. Acid-forming colonies on this medium appear yellow. After counting the acid-forming colonies, the proteolytic types were differentiated by flooding the plate with dilute acetic acid. These colonies are recognized by the clear halos surrounding them. The alkali-forming and inert group was enumerated by subtracting the sum of the acid-forming and proteolytic types from the total number of colonies. Plate cultures were incubated at 20° C. for 96 hours.

Titratable acidity was determined as percentage of lactic acid.

EXPERIMENTAL DATA

The average numbers of bacteria per cc., the average percentage of each bacterial group, and the average acidities in fresh milk and in the milk incubated at 70° F. for 24 and 48 hours are shown in table 1.

TABLE 1

Average numbers of bacteria per cc., average percentages of each bacterial group and average acidities in 75 corresponding samples of fresh raw and pasteurized milk and in raw and pasteurized milk incubated at 70° F.

TYPE OF MILK	BACTERIA PER CC.	PER CENT ACID FORMING	PER CENT PROTEOLYTIC	PER CENT ALKALI-FORMING & INERT	PER CENT ACID	PER CENT ACID COAGULATION
Fresh raw milk	14,800	29.40	7.00	63.60	0.16	—
Fresh pasteurized milk	2,070	18.80	5.00	76.20	0.16	—
Raw milk incubated 24 hours	241,000,000	92.10	0.55	7.35	0.26	0.0
Pasteurized milk incubated 24 hours	7,300,000	12.40	1.20	86.40	0.17	0.0
Raw milk incubated 48 hours	1,500,000,000	96.00	0.04	3.96	0.79	98.7
Pasteurized milk incubated 48 hours	960,000,000	76.10	0.20	23.70	0.55	57.3

DISCUSSION OF RESULTS

The initial bacterial counts of the 75 raw milk samples ranged from 2,400 to 50,000 per cc. Forty-four per cent contained less than 10,000 per cc. and 82.7 per cent gave counts of less than 25,000 per cc. The raw samples had an average count per cc. of 14,800 as compared to 2,070 for the freshly pasteurized samples.

Pasteurization resulted in an average reduction in bacterial numbers of 86 per cent, a decrease in the percentage of acid-forming bacteria from

29.4 to 18.8, a decrease in the percentage of the proteolytic group from 7.0 to 5.0 and an increase from 63.6 to 76.3 per cent in the alkali-forming and inert group.

After 24 hours of incubation the percentage of acid-forming bacteria in the raw and pasteurized samples of milk was 92.1 and 12.4, respectively. No significant difference occurred in the proportion of the proteolytic group in the two kinds of milk. The alkali-forming and inert group constituted 7.35 per cent of the raw milk flora as compared to 86.4 per cent of the flora of the pasteurized milk. Acid had not increased in any sample sufficiently to bring about coagulation. The flavors of the pasteurized samples, in all instances, were equal if not superior to the flavors of the corresponding raw samples. Many of the raw samples after 24 hours had developed sufficient acid to cause an undesirable flavor.

After 48 hours results were obtained that varied considerably from those secured after 24 hours. Less difference occurred in the percentages of the acid-forming and the alkali-forming and inert groups of the raw and pasteurized milk. The acid-forming bacteria constituted 96 per cent of the raw milk flora as compared to 76.1 per cent of the pasteurized milk flora. The alkali-forming and inert types of bacteria composed 3.96 and 23.7 per cent, respectively of the flora of the raw and pasteurized samples. Of the raw milk samples 98.7 per cent showed acid coagulation as compared to 57.3 per cent of the pasteurized samples. In 20 per cent of the pasteurized samples less than 0.4 per cent acid was present at the end of 48 hours. A pronounced bitter flavor had developed in all of the low acid samples and in a number of instances sweet curdling had taken place.

A period of delayed activity of the acid-forming bacteria followed by a period of increased activity was noted in the pasteurized samples. The proportion of these organisms increased from 12.4 to 76.1 per cent from the 24th to the 48th hour. In this same period a decrease occurred in the proportion of the alkali-forming and inert group. During the period in which the acid-producing organisms were relatively inactive there was an increased activity of the alkali-forming and inert group, probably accounting for the bitter flavor occurring in the samples of low acid content. Of the samples having less than 0.4 per cent acid at the end of 48 hours the alkali-forming and inert group of bacteria was in the majority, composing 52.4 per cent of the flora as compared to 46.7 per cent for the acid-forming organisms. After 72 hours the acid content of all these samples had increased to 0.6 per cent or more.

The results of this study show a considerable variation in details from those secured by Black *et al.* (4). This is to be expected since they were dealing with milk pasteurized in flasks and handled in such a way as to prevent subsequent contamination. However, the results support the findings of the above investigators as well as those of Thurston and Olson (6)

and furnish additional evidence that the spoilage of low count commercially pasteurized milk, in a great many instances, is due to the action of organisms other than the lactic acid producing types.

When pasteurized milk is consumed within 24 hours after delivery there is usually little danger of abnormal fermentations, such as encountered in this study, being noticeably present. When the product is kept an additional 12 to 24 hours at room temperature, as is often the case, pronounced abnormalities other than souring may be in evidence. If it is desired to overcome this condition, pure cultures of lactic acid producing organisms might be added to the pasteurized product prior to the bottling operation. Such a practice, however, would make it necessary to revise our present bacterial standards as the score of milk is determined largely by the number of organisms rather than by the types that are present.

SUMMARY AND CONCLUSIONS

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The results obtained in this study bear out the contention that, in many instances, organisms other than the lactic acid types are of major importance in the spoilage of commercially pasteurized milk derived from low count raw milk.

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American Dairy Science Association Announcements

ANNUAL MEETING
AMERICAN DAIRY SCIENCE ASSOCIATION

Ithaca and Geneva, New York
June 26 to 28, 1934

The first two days of the meeting will be held at Ithaca, and the third day (June 28) will be at Geneva. There will be conferences and demonstrations on judging dairy cattle and products in Ithaca on June 25, the day preceding the regular program. Rooms at a moderate rate will be furnished at Ithaca for the nights of June 25, 26, and 27. Further details will be published in the subsequent numbers of the JOURNAL OF DAIRY SCIENCE.

SCIENTIFIC PROGRAM

Members are invited to send titles of papers to the program committee. Papers will be limited in length to fifteen minutes. With the exception of invited speakers, at least one author of each paper must be a member of the American Dairy Science Association. Papers should represent original work not previously published.

The members of the program committee are Fordyce Ely, H. A. Ruehe, and J. M. Sherman, chairman. *Titles of papers must be received by the chairman of the program committee (Dairy Industry Building, Ithaca, N. Y.) before May 1.*

Members are asked to accept this invitation to send in papers as the official notice of the meeting, as individual notices will not be sent to members from the secretary's office.

NEWS ITEM

J. E. Dorman retired from the Bureau of Dairy Industry, U. S. Department of Agriculture at the close of the calendar year 1933 after more than 30 years in the Government service. His first connection with the Bureau was extension work in the South. Later he helped in the selection of the Beltsville Dairy Farm, Beltsville Md., and superintended the construction of its first buildings. Mr. Dorman was in charge of the Extension Service office of the Bureau of Dairy Industry, with headquarters in Salt Lake City, from 1913 to 1929. During the past 5 years he has been in charge of the summarization of records in the dairy herd-improvement association work with headquarters in Washington, D. C.

Mr. Dorman was one of the best known men in the Bureau, and one of the most respected men in the dairy industry. At the close of his last day in the Service, the Bureau held a reception in his honor at which Chief O. E. Reed gave an outline of the splendid service the Bureau had received from Mr. Dorman. Mr. Dorman responded in his characteristically humorous style.

Mr. Dorman expects to live in Florida. His fellow workers and friends wish him a long and happy life.